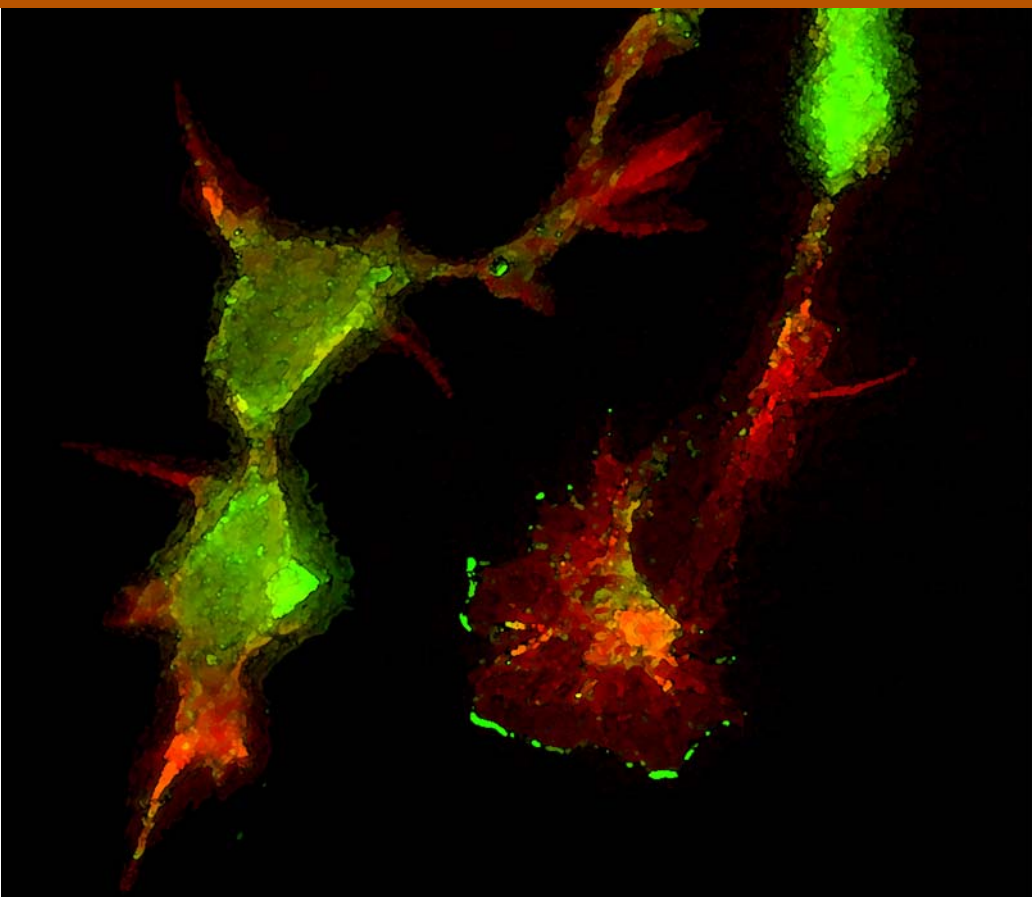
Abstracts of papers presented
at the 2010 meeting on

AXON GUIDANCE, SYNAPTIC PLASTICITY & REGENERATION

September 21–September 25, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

AXON GUIDANCE, SYNAPTIC PLASTICITY & REGENERATION

September 21–September 25, 2010

Arranged by

Graeme Davis, *University of California, San Francisco*
Alex Kolodkin, *HHMI / Johns Hopkins University Medical School*
Carol Mason, *Columbia University*

Cold Spring Harbor Laboratory
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Front Cover: Wild-type (right) and Rac1-knockout (left) cerebellar granule neurons co-stained with rhodamine-phalloidin (red) and an antibody against WAVE protein (green). The image has been processed using a watercolor filter. Image courtesy of Sabina Tahirovic and Frank Bradke, MPI of Neurobiology, Germany.

Back Cover: Confocal image of an adult mouse retina section, with labeling of the inner and outer plexiform layers using anti-vesicular glutamate transporter 1 (Red), the nuclei of retinal cells with Hoechst (Purple), and cholinergic amacrine cells with anti-Choline Acetyltransferase (Blue) to highlight their arborization in two sublaminae of the inner plexiform layer under rod bipolar cell end feet (stained with anti-protein kinase C alpha; Green). Image courtesy of Kim Nguyen-Ba-Charvet and Alain Chédotal, Institute de la Vision, Inserm, France.

AXON GUIDANCE, SYNAPTIC PLASTICITY & REGENERATION
Tuesday, September 21 – Saturday, September 25, 2010

Tuesday 7:30 pm	**1**	Axon to Synapse I
Wednesday 9:00 am	**2**	Synapse to Circuit I
Wednesday 2:00 pm	**3**	Poster Session I
Wednesday 4:30 pm		Wine and Cheese Party *
Wednesday 7:30 pm		Special Lecture
Thursday 9:00 am	**4**	Stem Cells, Regeneration and Disease I
Thursday 2:00 pm	**5**	Axon to Synapse II
Thursday 3:30 pm	**6**	Poster Session II
Thursday 7:30 pm	**7**	Synapse to Circuit II
Friday 9:00 am	**8**	Stem Cells, Regeneration and Disease II
Friday 2:00 pm		Special Lectures
Friday 3:30 pm	**9**	Poster Session III
Friday 6:00 pm		Concert
7:00 pm		Banquet
Saturday 9:00 am	**10**	Axon to Synapse III

Poster sessions are located in *Bush Lecture Hall*
* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:
Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm
Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

TUESDAY, September 21—7:30 PM

SESSION 1 AXON TO SYNAPSE I

Chairpersons: **K. Martin,** University of California, Los Angeles
Y. Zou, University of California, San Diego

WEDNESDAY, September 22—9:00 AM

SESSION 2 SYNAPSE TO CIRCUIT I

Chairpersons: **M. Feller,** University of California, Berkeley
A. Chedotal, Institut de la Vision, INSERM, Paris, France

WEDNESDAY, September 22—2:00 PM

SESSION 3 POSTER SESSION I

WEDNESDAY, September 22—4:30 PM

Wine and Cheese Party

WEDNESDAY, September 22—7:30 PM

SPECIAL LECTURE

Peter Devreotes
Johns Hopkins University School of Medicine

"The cell's compass—Chemoattractant bias of an excitable network of parallel signaling pathways directs cell migration"

THURSDAY, September 23—9:00 AM

SESSION 4 STEM CELLS, REGENERATION AND DISEASE I

Chairpersons: **Y. Jin,** Howard Hughes Medical Institute, University of California, San Diego
F. Bradke, Max Planck Institute of Neurobiology, Martinsried, Germany

THURSDAY, September 23—2:00 PM

SESSION 5 AXON TO SYNAPSE II

Chairpersons: **S. Arber,** Biozentrum, Basel, Switzerland
B. Sabatini, Harvard Medical School, Boston, Massachusetts

SESSION 7 SYNAPSE TO CIRCUIT II

Chairpersons: **J. Kaplan,** Massachusetts General Hospital, Harvard Medical School, Boston
G. Turrigiano, Brandeis University, Waltham, Massachusetts

Regulators of synaptic remodeling in *C. elegans* are revealed by identification of UNC-55 transcriptional targets
Sarah C. Petersen, Joseph D. Watson, Alyssa J. Fesmire, W W. Walthall, David M. Miller III.
Presenter affiliation: Vanderbilt University Medical Center, Nashville, Tennessee. 173

Otx2 homeoprotein transfer and signaling in visual cortex plasticity
Julien Spatazza, Marine Beurdeley, Ariel Di Nardo, Takao K. Hensch, Alain Prochiantz.
Presenter affiliation: CNRS UMR 7233, Paris, France. 174

FRIDAY, September 24—9:00 AM

SESSION 8 STEM CELLS, REGENERATION AND DISEASE II

Chairpersons: **Y. Zuo,** University of California, Santa Cruz
S. Temple, Albany Medical College, New York

Genes that promote or repress axon regrowth after laser surgery in *C. elegans*
Lizhen Chen, Zhiping Wang, Thomas Hubert, Anindya Ghosh-Roy, Zilu Wu, Sean O'Rourke, Bruce Bowerman, Yishi Jin, Andrew Chisholm.
Presenter affiliation: University of California San Diego, La Jolla, California. 175

Promoting axon growth over CNS inhibitors by targeting growth cone cytoskeletal compoments
Eun-Mi Hur, In Hong Yang, Justin Byun, Wen-Lin Xu, XXX Saijilafu, Nitish Thakor, Feng-Quan Zhou.
Presenter affiliation: Johns Hopkins University, Baltimore, Maryland. 176

PTEN deletion enhances the regenerative capacity of adult corticospinal neurons
Kai Liu, Yi Lu, Jae Lee, Ramsey Samara, Rafer Willenberg, Ilse Sears-Kraxberger, Andrea Tedeschi, Kevin Kyungsuk Park, Bin Cai, Bengang Xu, Lauren Connolly, Oswald Steward, Binhai Zheng, Zhigang He.
Presenter affiliation: Children's Hospital Boston/Harvard Medical School, Boston, Massachusetts. 177

FRIDAY, September 24—1:45 PM

SPECIAL LECTURES

Thomas Jessell
Howard Hughes Medical Institute
Columbia University

"The neurons and networks of spinal motor control"

Eve Marder
Brandeis University

"Compensation in robust network performance"

SESSION 9 POSTER SESSION III

Development of RNAi vectors eliciting cell type-specific, traceable gene knock down in the neural tube
Nicole H. Wilson, Esther T. Stoeckli.
Presenter affiliation: University of Zurich, Zurich, Switzerland. 231

ArfGAP1 is required for Semaphorin/Plexin signaling
Jim Wong, Marc Tessier-Lavigne.
Presenter affiliation: Genentech, Inc., South San Francisco, California. 232

The Coxsackievirus and Adenovirus Receptor (CAR) is involved in synaptic transmission.
Uta Wrackmeyer, Ulrike Pannasch, Dietmar Schmitz, Michael Gotthardt.
Presenter affiliation: Max-Delbrück-Center Berlin, Berlin, Germany. 233

The Plexin B receptor integrates both attractive and repulsive Semaphorin-mediated guidance in *Drosophila* to ensure accurate CNS circuitry assembly
Zhuhao Wu, Lora B. Sweeney, Joseph C. Ayoob, Kayam Chak, Benjamin J. Andreone, Rex Kerr, Liqun Luo, Marta Zlatic, Alex L. Kolodkin.
Presenter affiliation: Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland. 234

Degenerating tracts do not provide a strong guidance cue for regenerating optic axons—Observations in the *astray/robo2* mutant
Cameron Wyatt, Anselm Ebert, Michell M. Reimer, Kendall Rasband, Chi-Bin Chien, Thomas Becker, Catherina G. Becker.
Presenter affiliation: University of Edinburgh, Edinburgh, United Kingdom. 235

Highwire and downstream Wnd/JNK signaling regulate axonal outgrowth and branching in glutamatergic neurons in the brain
Xin Xiong, Richard Daniels, Catherine A. Collins.
Presenter affiliation: University of Michigan, Ann Arbor, Michigan. 236

FLRT2 and FLRT3 act as repulsive guidance cues for UNC5-positive neurons
Satoru Yamagishi, Falko Hampel, Katsuhiko Hata, Manuela Schwark, Elena Kvachnina, Martin Bastmeyer, Toshihide Yamashita, Victor Tarabykin, Joaquim Egea, Rüdiger Klein.
Presenter affiliation: Max Planck Institute of Neurobiology, Munich-Martinsried, Germany. 237

FRIDAY, September 24—6:00 PM

CONCERT
Grade Auditorium

Hahn-Bin, violin

Winner of the 2008-09 Young Concert Artists International Auditions, violinist Hahn-Bin makes his New York debut at Carnegie's Zankel Hall as recipient of the Peter Marino Concert Prize, as well as his Washington DC debut this season at the Kennedy Center's Terrace Theater. At the YCA Auditions, he was also awarded the Brownville Concert Series Prize and the Swiss Global Artistic Foundation Award for engagements in Europe.

Hahn-Bin made notable appearances with all of Korea's major orchestras, the Seoul, Bucheon, and Daejeon Philharmonics, in Korea and on tour in Japan. He has given recitals at the Louvre in Paris, and appeared as soloist with the Queensland Orchestra in Australia.

Hahn-Bin made his international debut at the age of twelve at the 42nd Grammy Awards' Salute to Classical Music, honoring Isaac Stern. Appearances with the Pacific and San Diego Symphonies followed quickly. At the age of sixteen, Hahn-Bin made his European debuts in a four-city tour with The State Youth Orchestra of Rheinland-Pfalz followed by performances in five cities across the U.S. with the orchestra. In 2005, Universal Music, Ltd. released his first CD, HAZE, with pianist John Blacklow, featuring works by Pärt, Janácek, Poulenc, Ravel, and Prokofiev.

Born in Seoul, Korea, in 1987, Hahn-Bin made his orchestral debut with the Seoul Philharmonic at the age of ten. The following year he moved to the U.S. to study with Robert Lipsett at the Colburn School in Los Angeles. Hahn-Bin earned his Diploma in 2009 from the Juilliard School, where he worked with Itzhak Perlman and Catherine Cho. He has been working with Mr. Perlman at the Perlman Music Program since 2002, and performed the Dvorak Piano Quintet with him at the Metropolitan Museum of Art in 2008. Hahn-Bin plays an 1825 J. F. Pressenda violin from the Mandell Collection of Southern California. In addition to playing the violin, Hahn-Bin works regularly on creating visual artworks and composing poetry.

FRIDAY, September 24

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SESSION 10 AXON TO SYNAPSE III

Chairpersons: **M. Halloran,** University of Wisconsin-Madison
T. Schwarz, Children's Hospital, Boston, Massachusetts

SLIT2/ROBO SIGNALING BETWEEN MOTOR NEURONS REGULATES AXON FASCICULATION

Alexander Jaworski[1], Natalie Kim[2], Steven J Burden[2], Marc Tessier-Lavigne[1]

[1]Genentech Inc., Division of Research, South San Francisco, CA, 94080, [2]New York University School of Medicine, Molecular Neurobiology Department, Skirball Institute of Biomolecular Medicine, New York, NY, 10016

As axons grow towards their targets, they often travel in groups, forming bundles of varying sizes. The ability of an axon to join and leave axonal fascicles in a regulated manner contributes to efficient pathfinding. However, the molecular cues regulating axon fasciculation within specific tracts remain incompletely characterized. Here we show that the axon guidance cue Slit2 and its receptors of the Robo family are expressed in motor axons, and that loss of *Slit2* or *Robo1* and *Robo2* in mice causes motor axons to prematurely defasciculate upon entering their target muscles. Interfering with Slit2 function in ventral spinal cord explants reduces the fasciculation of motor axons, whereas adding Slit2 can stimulate fasciculation, indicating that motor neuron-derived Slit2 acts to directly induce axon fasciculation in an autocrine or juxtaparacrine fashion. The non-receptor tyrosine kinase Abl has been implicated in Slit/Robo signaling, and we find that pharmacological inhibition of Abl reduces motor axon fasciculation *in vitro* and that *Abl* mutant mice display the same motor axon fasciculation phenotype as mice lacking *Slit2*. Our results reveal a novel function for Slit2/Robo signaling in maintaining motor axon fasciculation specifically during target innervation and suggest that Abl functions downstream of Slit2 in motor axons.

ROBO3 IS A DCC CO-RECEPTOR MEDIATING ATTRACTION OF PRECEREBELLAR NEURONS BY FLOOR PLATE

Pavol ZELINA, Yvrick ZAGAR, Alain CHEDOTAL

Vision Institute, INSERM U968, Paris, 75012, France

The Roundabout 3 (Robo3) receptor has been shown to play a key role in the development of spinal cord and hindbrain commissures. In Robo3 knockout (KO) mice, commissural axons and neurons are unable to cross the floor plate (FP) and instead project ipsilaterally. In the spinal cord, Robo3 seems to act by counteracting Robo1/Robo2 repulsion in precrossing axons in response to Slit repellents secreted by FP, thereby allowing axons to extend toward FP. However, in the hindbrain we showed that midline crossing by precerebellar axons (from the inferior olive and pons) is not rescued in Robo1/2/3 triple KO suggesting that in these neurons, Robo3 mechanism of action is different. Precerebellar axons express the DCC (Deleted in Colorectal Cancer) receptor and are attracted toward the floor plate by Netrin-1. In this study we tried to determine if Robo3 could co-operate with DCC to mediate attraction of precerebellar neurons by FP. We first showed that Robo3 and DCC could be co-immunoprecipitated from transfected 293 cells and E13 mouse hinbrain extracts. This interaction is mediated by their cytoplasmic domains and does not require Netrin-1. To assess the physiological relevance of this interaction in vivo, we crossed DCC and Robo3 KO mice. Interestingly, Robo3-/- ;DCC+/- embryos exhibit the same defects of precerebellar neuron development than DCC-/- KO mice. Moreover in Robo3-/- ;DCC-/- embryos, the ventral extension of precerebellar axons is completely abolished. This provides genetic evidence supporting a role for Robo3/DCC interaction in attracting precerebellar neurons toward the ventral midline. Next, rhombic lip (the origin of precerebellar neurons) explants from E13.5-E14.5 embryos were cultured in collagen gel with Netrin-1 expressing cells or FP explants. Precerebellar neurons were identified by immunostaining (Pax6, Robo3, DCC) and using in utero electroporation of GFP. In wild type explants, precerebellar neurons extended processes and migrated toward FP and Netrin-1 expressing cells. By contrast, attraction appears to be inhibited with explants derived from Robo3-/- embryos. Altogether, these results suggest that Robo3 forms a receptor complex with DCC that mediates the attraction of precerebellar axons and neurons toward the FP.

THE REFINEMENT OF SPINAL MOTOR AXON GUIDANCE BY EPHRIN-MEDIATED CIS-ATTENUATION OF EPHRIN:EPH FORWARD SIGNALLING

Tzu-Jen Kao[1], Artur Kania[1,2,3]

[1]Institut de recherches cliniques de Montréal, Neural Circuit Development, Montréal, H2W 1R7, Canada, [2]McGill University, Anatomy and Cell Biology, Montréal, H3A 2B2, Canada, [3]Université de Montréal, Faculté de Médecine, Montréal, H3C 3J7, Canada

Eph tyrosine kinase receptors and their ephrin ligands are co-expressed on neuronal growth cones raising questions about their relative contribution to axon guidance. Two divergent models of ephrin function in growth cones have been proposed: (1) ephrins function as axonal receptors and interact with target cell Ephs leading to Eph:ephrin reverse signalling *"in trans"* or (2) ephrins bind to Eph receptors co-expressed in the same growth cone and attenuate *"in cis"* their forward ephrin:Eph signalling. We are aiming to resolve between these alternatives in the context of spinal lateral motor column (LMC) motor axon innervation of limb muscles. EphA4 and EphB1 receptors are restricted to subpopulations of LMC motor neurons and mediate the selection of their axonal trajectories in response to limb mesenchyme ephrins. However, several other Eph receptors are expressed at low levels by most LMC neurons while specific ephrin ligands are enriched in LMC subpopulations. Although *in vitro* evidence suggests that reverse Eph:ephrin signalling contributes to LMC axon guidance, we sought to examine *in vivo*, whether ephrins expressed by LMC axons can attenuate Eph signalling *"in cis"*.

Ectopic expression of ephrin-A5 and ephrin-B2 in LMC neurons and LMC-specific siRNA knockdown of ephrin-A5 and ephrin-B2 by *in ovo* electroporation lead to LMC axon misrouting consistent with ephrins attenuating Eph signalling *"in cis"*. Expression of an ephrin-A5 mutant unable to bind to EphAs *"in trans"* or an ephrin-B2 mutant lacking the intracellular domain results in the same LMC axonal re-direction phenotypes as over-expression of wild-type ephrins, suggesting that reverse Eph:ephrin signalling is not required for this effect. In addition, *in vitro* responses to ephrin stripes of LMC axons with a loss or gain of ephrin function also indicate that ephrins can modulate Eph receptor function *"in cis"* in motor axons.

Our observations argue that *cis*-attenuation of ephrin:Eph forward signalling contributes to the fidelity of motor axon trajectory selection. We propose that in addition to Eph:ephrins signalling *"in trans"*, ephrin expression in axons modulates *"in cis"* the sensitivity of Eph receptors to exogenous ephrins and thus increases the diversity of Eph signalling responses in axon guidance.

NOVEL ROLES OF F-BAR PROTEINS IN GROWTH CONE MORPHOLOGY, AXONAL OUTGROWTH AND BRANCHING

Witchuda Saengsawang, Thomas Fothergill, Chris Viesselmann, Jason Ballweg, Derek C Lumbard, Erik W Dent

University of Wisconsin-Madison, Department of Anatomy, Madison, WI, 53706

The microtubule and the actin cytoskeleton in growth cones are crucial for axonal outgrowth, branching and guidance. They are continuously remodeled in response to multiple guidance cues, but little is known about the proteins that regulate the cytoskeleton during these important events in axon development. We investigated the function of two putative cytoskeletal regulatory proteins, Cdc42-interacting protein 4 (CIP4) and formin-binding protein 17 (FBP17), in CNS axon development. CIP4 and FBP17 are members of CIP4 subfamily of F-BAR proteins. F-BAR proteins are believed to function in membrane deformation by inducing concave membrane curvature; a key event in endocytosis and membrane tubulization. CIP4 and FBP17 interact with multiple partners, including membrane phospholipids (PS and PIP2), active Cdc42, actin-associated proteins (N-WASP and mDia) and microtubules (CIP4 only). We discovered FBP17 is expressed throughout development in cortex and hippocampus, whereas CIP4 is expressed prenatally and declines to low levels soon after birth, suggesting that CIP4 could serve an important function in early axon outgrowth and guidance. CIP4 and FBP17 also have distinct localization patterns in embryonic cortical neurons. CIP4 co-localizes with both tyrosinated microtubules and f-actin in the growth cone while FBP17 strongly labels growth cone f-actin bundles. In addition, time-lapse imaging of both proteins with total internal reflection fluorescence (TIRF) microscopy showed they concentrated at the tips of extending growth cone filopodia and lamellipodia. Overexpression of CIP4 and FBP17 significantly decreased both axon length and branch number, consistent with a possible role in endocytic function. Surprisingly, we observed that neurons overexpressing CIP4 develop large lamellar growth cones, with CIP4 concentrated along their leading edge. Such growth cone enlargement and leading edge concentration is potentially inconsistent with increased endocytosis and membrane tubulization. Rather, in neurons, these proteins may function primarily in filopodial and lamellipodial extension, similar to what has been reported for another member of the F-BAR family of proteins, srGAP2 (Guerrier et al., 2009). Ongoing RNAi experiments will test this hypothesis.

ZIPCODE BINDING PROTEIN 1 REGULATES B-ACTIN MRNA TRANSPORT, LOCAL TRANSLATION AND AXON GUIDANCE.

Kristy Welshhans, Gary J Bassell

Emory University School of Medicine, Department of Cell Biology, Atlanta, GA, 30322

An important mechanism underlying axon guidance is the local protein synthesis of β-actin in growth cones of developing axons *in vitro*. The localization of β-actin mRNA is regulated by the binding of an mRNA binding protein, zipcode binding protein 1 (ZBP1), to a 54 nucleotide sequence within its 3' untranslated region, although the requirement of ZBP1 for local translation has not been shown. Previous work in neuroblastoma cells has shown that the binding of ZBP1 to β-actin mRNA results in translational repression, which can be relieved by Src phosphorylation of ZBP1 at Try396, suggesting a possible mechanism to regulate mRNA translation locally. The requirement of ZBP1 or any mRNA binding protein in local mRNA translation has not been previously studied. As such, we investigated the role of ZBP1 in the localization and translation of β-actin mRNA within the axon and examined the functional relevance of this localization for growth cone motility and guidance. The role of ZBP1 was investigated using both primary cortical neurons from ZBP1 knockout mice and overexpression of a non-phosphorylatable mutant form of ZBP1 in wild-type neurons. We find that although ZBP1 does not appear to regulate basal axon outgrowth, it does regulate aspects of filopodial dynamics. To investigate the role of ZBP1 in axon guidance, we applied an *in vitro* turning assay to primary cortical neurons and demonstrate their attraction to either netrin-1 or brain derived neurotrophic factor (BDNF). Importantly, both netrin-1- and BDNF-induced attractive turning was protein synthesis dependent. Additionally, ZBP1 knockout neurons or those neurons overexpressing a non-phosphorylatable mutant of ZBP1 lose this attractive turning response. Furthermore, the BDNF-stimulated localization of β-actin mRNA and enrichment of β-actin protein in growth cones was reduced in ZBP1 knockout neurons. We are now using a photoconvertible translation reporter to investigate the role of ZBP1 in the local translation of β-actin mRNA in the growth cone. Taken together, these results suggest that local phosphorylation of ZBP1 by Src kinase results in local translation of β-actin mRNA, thereby regulating multiple aspects of growth cone motility, including growth cone guidance. Furthermore, this study suggests a novel role for the mRNA binding protein ZBP1 as a regulator of axon guidance. Overall, the current work describes a basic mechanism that may be essential to proper development of the nervous system.

ANALYSIS OF THE ROLE OF LIM-HD TRANSCRIPTION FACTORS IN DEFINING AXON TRAJECTORIES *IN VIVO*

Namrata S Asuri[1,2], Erica F Andersen[1,2], Mary C Halloran[1]

[1]University of Wisconsin- Madison, Departments of Zoology and Anatomy, Madison, WI, 53706, [2]University of Wisconsin- Madison, Genetics Training Program, Madison, WI, 53706

The establishment of proper neuronal morphology and accurate axon pathfinding are crucial for the formation of a functional nervous system. Several studies have shown that transcription factors can not only define cell fates but also direct final axon trajectories. However, little is known about the molecules regulated by these transcription factors that ultimately control axon motility and pathfinding. We are investigating this question in zebrafish Rohon-Beard (RB) and trigeminal somatosensory neurons. These neurons have stereotyped axon projection patterns that are regulated in part by LIM-HD transcription factors. Inhibition of LIM-HD transcription factors using a dominant negative cofactor of LIM (DN-CLIM) eliminates most peripheral axons without affecting central axons of these neurons. We are using two approaches to understand how these transcription factors control peripheral axon formation and guidance. First, we are imaging growth cone behaviors and F-actin to determine which motile behaviors are affected. We are using a new biosensor to visualize F-actin in developing sensory neurons *in vivo*. Live imaging of actin dynamics in wildtype embryos shows a strong accumulation of F-actin signal at the site of peripheral axon emergence prior to its initiation. In DN-CLIM expressing embryos filopodial protrusions and F-actin accumulation can occur, but peripheral axons fail to extend. These results suggest that LIM-HD molecules do not affect the ability of F-actin to accumulate but perhaps regulate a later event in axon formation.
Second, we are using microarray analysis to identify genes regulated by LIM-HD transcription factors that function in peripheral axon outgrowth. We compared gene expression between wildtype and DN-CLIM expressing embryos and identified 569 transcripts ($p < 0.05$) showing differential expression. In addition to axon guidance receptors, we have also identified cell adhesion molecules, cytoskeletal regulators, membrane trafficking molecules and novel genes. We are currently testing the function of candidate genes with morpholino knockdown. Preliminary analysis of one of these, a novel gene containing tubulin binding domains suggests it is required for the guidance of peripheral trigeminal axons. Together, these two approaches are aimed at understanding the mechanisms by which transcription factors control axon trajectories.

LIPID-MEDIATED AXON GUIDANCE IN THE DEVELOPING SPINAL CORD

Adam T Guy[1], Yasuko Nagatsuka[2], Peter Greimel[3], Takuji Nabetani[3], Yukishige Ito[4], Kunihiro Ohta[5,6], Yoshio Hirabayashi[2,7], Hiroyuki Kamiguchi[1]

[1]Neuronal Growth Mechanisms Laboratory, RIKEN Brain Science Institute, Wakoh, Saitama, 351-0198, Japan, [2]Molecular Membrane Neuroscience Laboratory, RIKEN Brain Science Institute, Wakoh, Saitama, 351-0198, Japan, [3]Research Group for Lipid Dynamics, RIKEN Advanced Science Institute, Wakoh, Saitama, 351-0198, Japan, [4]Synthetic Cellular Chemistry Laboratory, RIKEN Advanced Science Institute, Wakoh, Saitama, 351-0198, Japan, [5]Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, 153-8902, Japan, [6]Chiome Bioscience Inc., RIKEN-WAKO Incubation Plaza Suite 203, Wakoh, Saitama, 351-0104, Japan, [7]CREST Japan Science and Technology Agency, Kawaguchi Center Building, Kawaguchi, Saitama, 332-0012, Japan

To date many mechanisms in neural development have been described based on axon guidance mediated by proteins and their derivatives. In contrast, we have identified a novel mechanism for early patterning of dorsal root ganglion (DRG) primary sensory afferents in the spinal cord that is mediated by lyso-phosphatidylglucoside (Lyso-PtdGlc), a water-soluble derivative of the membrane lipid phosphatidyl-β-D-glucoside (PtdGlc). PtdGlc is synthesised by immature glial cells and released as Lyso-PtdGlc into the extracellular space following phospholipase-mediated hydrolysis of the *sn*-2 acyl chain of PtdGlc. Sensory DRG afferents in the spinal cord can be broadly divided into two types: those from NGF-dependent, TrkA-expressing neurons, and those that are NT-3 dependent and express TrkC. At the developmental stage when DRG afferents have entered the dorsal spinal cord, PtdGlc is concentrated in the dorsal white matter, where it co-localises with axons that express TrkC but not with those that are TrkA-positive. It was found that *in vitro*, Lyso-PtdGlc is a potent axon chemorepellent specific for NGF-dependent but not NT3-dependent DRG sensory neurons, both at the level of dissociated single cells and in organotypic tissue culture explants in a 3-D collagen gel matrix. Loss-of-function of Lyso-PtdGlc *in vivo* in the developing spinal cord causes ectopic TrkA-expressing axon projection into the dorsal white matter PtdGlc domain, where normally TrkC-expressing axons predominate. This study demonstrates that in the spinal cord white matter, the two major sensory afferents are sorted and patterned by their sensitivity to the chemorepellent Lyso-PtdGlc.

GATA3 REGULATES THE INITIATION OF AUDITORY CIRCUIT ASSEMBLY

Jessica M Appler, Cindy Lu, Edmund J Koundakjian, Lisa V Goodrich

Harvard Medical School, Department of Neurobiology, Boston, MA, 02115

During development, precisely organized neural circuits emerge through a series of exquisitely timed events. We manipulated the timing of neural circuit formation in the auditory system by disrupting the expression of the transcription factor GATA3. The primary neurons for the sense of hearing are spiral ganglion neurons, which receive input from hair cells in the cochlea and communicate all aspects of complex sound stimuli to neurons in the cochlear nucleus. Auditory circuit assembly begins when spiral ganglion neurons arise together with vestibular ganglion neurons within a common neurogenic domain. One of the earliest signs of the segregation of these two neuronal populations is the restriction of the expression of the transcription factor GATA3 to spiral ganglion neurons. In other organ systems, GATA factors are master regulators of cell fate and cooperate with other transcription factors to regulate cell-type specific aspects of differentiation. Therefore, we hypothesized that GATA3 guides the integration of spiral ganglion neurons into functional auditory circuits.

Our investigations indicate that GATA3 regulates the timing of differentiation in spiral ganglion neurons. We generated mice where *GATA3* was selectively removed from neurons using a *BhlhB5-Cre* driver. In *GATA3* conditional knock-outs, spiral ganglion neurons prematurely extend their processes into the cochlear duct towards the developing sensory epithelium. This precocious outgrowth is accompanied by accelerated changes in gene expression, including ectopic expression of guidance molecules that may contribute to subsequent wiring defects. *GATA3* is mutated in the human deafness syndrome hypoparathyroidism, sensorineural deafness, and renal anomalies (HDR), but the origin of deafness is unclear. By elucidating the role of GATA3 in spiral ganglion neurons, we can gain insights into the etiology of deafness in HDR patients and better understand the molecular mechanisms that underlie brain wiring.

CELL-SURFACE MOLECULES SPECIFY SYNAPTIC-LAYER TARGETING IN THE DROSOPHILA VISUAL SYSTEM

Sandra Berger-Mueller*, Satoko Hakeda-Suzuki*, Klaudiusz Mann, Tatiana Tomasi, Takashi Suzuki

Max Planck Institute of Neurobiology, Axon Guidance and Neuronal Connectivity, Martinsried, 82152, Germany

*These authors contributed equally to this work.

Information-processing in the brain is critically dependent on precise synaptic communication between specific neurons. Synaptic connections are often organized in layered structures, each layer containing synapses between neurons that have similar functional properties. For instance, in the Drosophila visual system, two types of photoreceptor cells (R7 and R8) which detect different wavelengths form synapses in distinct layers in the medulla (M6 and M3, respectively). Although several axon guidance and cell adhesion molecules have been shown to be required for the layer targeting of photoreceptor axons, the underlying molecular mechanisms are still unclear.
The protocadherin Flamingo (Fmi) and the transmembrane receptor Golden goal (Gogo) have a similar phenotype in R8 axon targeting. We show that Gogo physically interacts with Fmi in cis, and that Gogo localization at the photoreceptor growth cone is largely dependent on Fmi. Loss-of-function and gain-of-function interaction studies indicate that the Gogo-Fmi complex directs R8 photoreceptor axons to the correct synaptic layer M3, whereas Gogo alone promotes adhesion to the temporary target layer M1. Moreover, the Gogo-Fmi complex mediates intracellular signaling through Gogo cytoplasmic domain, and Gogo signaling is regulated by the phosphorylation of a conserved tyrosine in the cytoplasmic domain. Fmi is also required in a subset of target cells for R8 axon targeting, suggesting Fmi homophilic interactions between photoreceptor axons and their targets. Altogether, we propose that the dynamic regulation of the Gogo-Fmi complex formation specifies synaptic-layer selection of photoreceptors, and that a combinatorial code of cell-surface molecules may be a key mechanism in synaptic-layer targeting.

ACTIVITY-DEPENDENT *DE NOVO* SPINE FORMATION

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Cortical pyramidal neurons receive excitatory inputs onto small protrusions emanating from their dendrite called spines. Each spine undergoes activity-dependent remodeling, stabilization, and pruning process during development, and many studies have demonstrated that these structural changes can be triggered by learning and experience. However, the trigger and pathways that determine initial spine growth are largely unknown. We have developed an approach to induce and monitor *de novo* spine formation in real-time using combined two-photon laser-scanning microscopy and two-photon laser uncaging of glutamate. Our data demonstrate that glutamate is sufficient to trigger *de novo* spine growth from the dendrite shaft in a location-specific manner. We find that spinogenesis requires Ca2+ influx through NMDARs and activation of PKA but is independent of CAMKII and TrkB receptors. Furthermore, activity in existing spines increases the probability of forming new spines in the vicinity of the active spine. These results demonstrate that neural circuit formation is modified by activity in a spatially precise and cooperative manner.

VISUAL EXPERIENCE MODULATES SPATIO-TEMPORAL PATTERNS OF CIRCUIT ACTIVATION

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Reduction in sensory drive during early development can alter significantly the ability of neurons to respond to environmental stimuli. In the visual cortex changes in sensory responsiveness are induced very rapidly when the sensory input is altered unilaterally early in postnatal development. Several models have been proposed to relate the functional changes recorded in vivo to specific forms of plasticity or to other cellular and molecular mechanisms. While approaches that dissect the circuit out in its components have been extremely successful in providing a pristine map of all the modifications at different sites of cortical networks, a more synthetic picture can only be obtained by taking a bird's eye view of how cortical areas integrate and propagate signals. Here we combined voltage sensitive dyes (VSD) optical imaging and electrophysiology recordings to determine the effects of monocular deprivation (MD) on the flow of electrical signals within and between the superficial layers of rodent primary visual cortex (V1). Our data show that MD decreases the spatio-temporal patterns of activation of L4 (by 29.4 ± 3.2 %; two way ANOVA: $p < 0.05$; Control: $n = 10$; Deprived: $n = 9$) and L2/3 (by 37.3 ± 2.5 %; two way ANOVA: $p < 0.001$). The ratio of L2/3 activation versus L4 remained unchanged (Control: 1.2 ± 0.1; Deprived: 1.3 ± 0.3; $p=0.8$) suggesting a direct correlation between the reduction of L4 activation and that of L2/3. The mechanism regulating the reduced activation is an MD-dependent increase in inhibitory drive in the input layer of V1, which results in a reduction of the feedforward propagation of signals. The horizontal propagation of signals elicited by direct stimulation of L2/3 is not affected by MD, in agreement with previous data suggesting L2/3 as the site of homeostatic plasticity during the critical period for visual cortical plasticity. The results presented here show that experience-dependent changes in visual responsiveness may emerge from an unbalance between feedforward and feedback circuit activation determined by the integration several different forms of plasticity at different sites of the cortical circuit.

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HTS/ADDUCIN COORDINATES SYNAPTIC ELABORATION AND ELIMINATION

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Neural development requires both synapse elaboration and elimination, yet relatively little is known about how these opposing activities are coordinated. Here we provide evidence Hts/Adducin can serve this function. We show that Drosophila Hts/Adducin is highly expressed both pre- and postsynaptically at the Drosophila NMJ. We then demonstrate that presynaptic Hts/Adducin is necessary and sufficient to control two opposing processes: 1) synapse stabilization as determined by light level, ultrastructural and electrophysiological assays and 2) the elaboration of actin-based, filopodia-like protrusions that drive synaptogenesis and promote nerve terminal growth. Mechanistically, Hts/Adducin has actin-capping activity, a relatively unexplored process during presynaptic development. We propose that phosphorylation-dependent dissociation of Hts/Adducin from the submembranous, presynaptic spectrin skeleton simultaneously destabilizes the NMJ and promotes actin-based filopodial membrane protrusion. The net effect is an expansion of the nerve terminal despite existing synaptic instability. Thus, Hts/Adducin may define a mechanism to switch between synapse stability and dynamics.

HBL-1 PATTERNS SYNAPTIC REMODELING IN *C. ELEGANS*.

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Synapses are added and removed from circuits throughout the life of an animal. Although synaptic refinement plays a pivotal role in circuit and likely cognitive development, little is known about the genetic pathways governing this process. In *C. elegans*, the GABAergic DD motor neurons undergo developmentally programmed remodeling, whereby synapses onto ventral body muscles are eliminated and replaced with dorsal synapses. Here we show that DD remodeling is extensively patterned and we identify the transcription factor HBL-1 as a central regulator of this process. DD remodeling is activity-dependent and remodeled synapses form in a proximal to distal spatial pattern. Remodeling is restricted to the DD neurons by UNC-55, which represses *hbl-1* expression in other neurons. Mutants lacking HBL-1 have delayed DD remodeling whereas precocious remodeling is observed in mutants lacking the microRNA *mir-84*, which inhibits translation of the *hbl-1* mRNA. Thus, several regulatory factors converge on *hbl-1*, thereby patterning DD plasticity.

CADHERIN-9 REGULATES INPUT-SPECIFIC SYNAPSE FORMATION IN THE DEVELOPING HIPPOCAMPUS

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A central organizing feature of the mammalian brain is the development of specific synaptic connections. Here we show that hippocampal neurons grown in culture develop synaptic diversity that is remarkably similar to the brain. We found that cultured dentate gyrus (DG) neurons preferentially form appropriate connections with CA3 neurons rather than DG or CA1 neurons and that these DG-CA3 mossy fiber synapses develop independent of activity and positional cues. Instead, formation of DG mossy fiber synapses depends on expression of the specific molecular cue Cadherin-9. Cadherin-9 is a type II cadherin selectively expressed by DG and CA3 neurons that undergoes homophilic binding and recruits the signaling molecule β-catenin. In culture, Cadherin-9 is required specifically for the development of DG-CA3 mossy fiber synapses but not other types of excitatory synapses. In vivo, we show that decreasing Cadherin-9 expression from either DG or CA3 neurons severely disrupts the normal development of DG-CA3 mossy fiber synapses. Taken together, our data suggest that homophilic Cadherin-9 interactions simultaneously transmit synaptogenic signals in both pre- and post-synaptic cells to coordinate DG mossy fiber synapse formation.

IDENTIFICATION OF AN ASTROCYTE SECRETED PROTEIN THAT IS SUFFICIENT TO INDUCE FULLY FUNCTIONAL SYNAPSE FORMATION

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Synapses are specialized cell adhesions that are the fundamental functional units of the nervous system, but the extracellular signals that induce CNS synapse formation and function are poorly understood. We have been investigating the role of astrocytes in the formation and function of excitatory synapses *in vitro* and *in vivo*. Using retinal ganglion cells (RGCs) as a model CNS neuron, we previously found that astrocytes release several factors that strongly enhance the formation and function of excitatory synapses onto RGCs. We identified thrombospondins as astrocyte-derived proteins that normally help to promote CNS synaptogenesis *in vivo* and are sufficient to induce ultrastructurally normal synapses *in vitro*, however we found that thrombospondin-induced synapses are postsynaptically silent, lacking AMPA glutamate receptors. The AMPA subtype of glutamate receptor mediates fast excitatory synaptic transmission, and regulated trafficking of AMPA receptors is an important mechanism for controlling synaptic strength. We therefore set out to identify the additional activity-inducing factor being released by astrocytes. Initial 2-D electrophoresis experiments showed that astrocytes secrete hundreds of proteins in varying abundances, therefore we used a combination of chromatography columns to fractionate the proteins present in astrocyte conditioned medium (ACM), and obtained a fraction that contained 1% of the starting proteins present in ACM and was 5-fold enriched for functional activity. Analysis of this fraction by mass spectrometry identified approximately 20 proteins as being present, and using an over-expression system we identified one of these proteins as being sufficient to induce an increase in synaptic activity between RGCs. Addition of this purified protein to RGCs *in vitro* causes an increase in the surface levels of AMPA glutamate receptors, and a clustering of these receptors in synaptic sites. We are currently investigating the role of this protein in synapse formation and strengthening *in vivo*.

ANATOMICAL PLASTICITY OF DENDRITIC SPINES IN THE ADULT CEREBRAL CORTEX IS RESTRICTED BY NGR1

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In completed studies, we have shown that blockade of myelin inhibitor action by NgR1 decoy or by gene deletion partially overcomes myelin inhibition of axonal growth in vitro, and supports a significant degree of axon plasticity, sprouting and regeneration after spinal cord hemisection, spinal contusion, pyramidotomy, optic nerve crush and stroke(1-7). Moreover, electrophysiological plasticity of visual cortex ocular dominance is maintained at critical period levels far into adulthood when NgR1 is absent(8). However, NgR1's role in the anatomical stability of synapses of the adult brain is unknown.

Here we use chronic in vivo imaging to assess the role of NgR1 in limiting structural plasticity in vivo. Apical tufts of L5 pyramidal cells were imaged in M1 of P180 thy1-EGFP-M mice at two-time points over 14 days. Dendritic spines in NgR1 null animals are significantly less likely to persist over the 14 day period when compared to heterozygotes (0.649 + 0.017, 0.85 + 0.009, $p<.001$). To evaluate whether or not NgR1 regulates transient turnover, we imaged apical tufts of L5 pyramidal cells in thy1-YFP-H animals every 4 days for at least 16 days. In order to determine whether this is a pre or post-synaptic action of NgR1, we have adapted a chambered culture system (9) to track dendritic spines and axonal varicosities in cultured neurons in vitro. Finally, to evaluate if this is a developmental or regulatory role of NgR1, we imaged mice with conditional NgR1 deletions for 16 days before and after conditional NgR1 deletion. We are now assessing the interaction of NgR1-/- dependent spine turnover with experience-dependent spine turnover in the somatosensory cortex.

These studies delineate a role for NgR1 in limiting the anatomical stability of the adult brain at the level of synaptic structures.

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A BIOINFORMATIC AND *IN SITU* SCREEN FOR NOVEL AXON GUIDANCE MOLECULES

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Sophisticated genetic and biochemical screens for axon guidance molecules have identified a number of ligands and their axonal receptors. Many of these fall into a few major classes that share a limited number of structural motifs which are conserved from invertebrates to vertebrates. Although it has been speculated that the majority of axon guidance molecules have been discovered it is unlikely that sufficient molecules have been identified to encode the complete wiring of the embryonic nervous system.

In order to identify further axon guidance molecules we have taken a systematic bioinformatic approach to identify novel transmembrane proteins that contain any of a number of conserved extracellular protein domains found in known axon guidance molecules. This screen has identified 162 genes in *Drosophila melanogaster* that fulfil these criteria and their expression patterns were subsequently determined by *in situ* hybridization. This study yielded 41 candidates that show neural expression in the embryo during the period of axon extension. These include 9 genes that have orthologues in vertebrates including the CG32635/Neto and Ten/Odz families, Gogo and CG8403/Pikachurin.

Pikachurin is a type II transmembrane receptor-like protein with predicted EGF and laminin domains. It has been identified to interact with dystroglycan and have a role in positioning the bipolar cell dendrites in the mouse photoreceptor ribbon synapse (Sato et al 2008). *Drosophila* Pikachurin is widely expressed in the embryonic nervous system. A small deletion in pikachurin created by imprecise excision of an existing P-element reveals a likely role in the targeting of the ISNb motor neurons.

IMAGING CENTROSOME POSITIONING DURING AXON FORMATION *IN VIVO*

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Neurons must develop highly specialized morphologies for proper nervous system connectivity. The establishment of polarity and the formation of distinct cellular processes are fundamental to precise neuronal structuring. The stereotyped development of the zebrafish Rohon-Beard (RB) spinal sensory neurons provides an ideal system to study the mechanisms controlling neuronal morphogenesis *in vivo*. RBs first extend ascending and descending central axons within the spinal cord, followed by a single peripheral axon that forms either off the cell body or as a branch off of one of the central axons, then exits the spinal cord to innervate the skin. The peripheral axon is a defining characteristic of RB neuron morphology, yet little is known about what regulates the formation of this distinct process or what distinguishes it from the central axons. The centrosome is known for its roles as a microtubule-organizing center and in establishing cell polarity, however its function in axon development is controversial. The centrosome has been implicated in axon specification in cultured hippocampal neurons, but other studies suggest that the centrosome is dispensable for axon formation. We hypothesize that the centrosome plays a role in RB neuron morphogenesis, specifically in peripheral axon formation. We use confocal time-lapse imaging to observe the dynamics of centrosome localization within developing RB neurons *in vivo*. Results show a correlation between centrosome and peripheral axon positioning, suggesting that the centrosome determines the site of peripheral axon formation. The activity of LIM-homeodomain (HD) containing transcription factors was previously shown to be necessary for peripheral axon development, but the mechanisms by which they act are unknown. We are currently exploring whether there is a link between LIM-HD activity and centrosome positioning.

A ROLE FOR ERM PROTEIN ACTIVATION IN NETRIN-1/DCC-MEDIATED AXON OUTGROWTH

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The responsiveness of the growth cone to guidance cues relies on its capacity to translate attractive and repulsive signals into cellular movements which are regulated by dramatic cytoskeletal rearrangements. *Deleted in Colorectal Cancer* (DCC) is one of the receptors involved in the attractive growth response downstream of the chemotropic protein Netrin-1, a highly conserved axon guidance cue. Upon Netrin-1 stimulation, DCC is phosphorylated and mediates the assembly of intracellular signalling complexes within the growth cone, leading to the activation of actin cytoskeleton regulators: the Rho GTPases Rac1 and Cdc42.

Here we investigate the role of F-actin-binding ERM proteins (Ezrin-Radixin-Moesin) in the context of Netrin-1/DCC-mediated axon outgrowth. We found ERM proteins to form a complex with DCC in rat embryonic brains by mass spectrometry. The formation of a DCC-ezrin complex was confirmed by GST pull-down assays and co-immunoprecipitation in mouse neuroblastoma cells and embryonic rat cortical neurons. We show that ERM proteins are activated downstream of Netrin-1 and DCC and that Rho GTPases appear to be involved in the phosphorylation-dependent activation of ERM proteins downstream of Netrin-1. We observed that activated ERM proteins accumulate in filopodia where they colocalize with DCC. In a Netrin-1-dependent axon outgrowth assay, we found that the expression of DCC and inactive mutants of ezrin significantly decreases axon outgrowth downstream of Netrin-1. In summary, these results show that Netrin-1 regulates the formation of a DCC-ezrin complex which accumulates in filopodia and that the F-actin-binding activity of ezrin is required for Netrin-1-mediated axon outgrowth.

TRANSCRIPTIONAL REGULATION OF UNC-5 BY THE GATA TRANSCRIPTION FACTOR, GRN, DURING MOTONEURON SPECIFICATION IN DROSOPHILA.

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Development of the CNS is driven by the establishment of complex patterns of gene expression at precise times and spatial locations. During motoneuron specification, neurons acquire a unique set of surface molecules essential for processes such as axon guidance and target recognition. Transcription factors play a central role by regulating the expression of these cell-specific surface molecules. Previous work in our lab has revealed that a transcription factor, eve, acts to positively regulate the expression of a key guidance molecule Unc-5, in dorsal projecting motoneurons (dMNs) in Drosophila embryos. However, since eve is a transcriptional repressor, it is unlikely that it directly regulates expression of Unc-5. In our study to identify direct regulators of Unc-5, we have defined a 4 kB region at the Unc-5 locus which recapitulates Unc-5 expression in the ventral nerve cord. Bioinformatic analysis of this promoter region identifies GATA consensus motifs conserved across all 12 Drosophila species. Interestingly, expression of grn, a GATA transcription factor whose expression in dMNs is dependent on eve, results in an increase in transcriptional activity in an in vitro Unc-5 reporter assay. Genetic studies also support a role for grn in positively regulating Unc-5 expression. We observe a strong transheterozygous interaction between grn and Unc-5 heterozygous mutants. Upon ectopic expression of grn in dMP2 motoneurons, a set of gut innervating motoneurons that don't normally express either grn or Unc-5, we observe upregulation of Unc-5 expression. Furthermore, ectopic expression of either grn or Unc-5, results in axonal exit of dMP2 neurons away from the gut towards the muscle field. Taken together, our data supports a role for grn in positively regulating Unc-5 in dMNs, and importantly provides a direct functional link between the transcriptional regulatory network and a specific axon guidance receptor in a specific set of motoneurons during MN specification.

DECIPHERING THE MECHANISMS OF DORSAL MOTONEURON SPECIFICATION AND AXOGENESIS IN DROSOPHILA MELANOGASTER USING MRNA PROFILING.

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How the axon of an individual neuron makes different decisions during pathfinding is dependent on the surface receptors for guidance molecules expressed on its membrane. To date, numerous receptors have been identified, however their precise expression on individual motoneurons as well as how they are regulated is not known.
To elucidate how transcriptional codes regulate the expression of surface molecules we use as a model two Drosophila dorsal motor neurons (dMNs), aCC and RP2. This system provides several advantages: 1-RP2 and aCC can be individually labeled and isolated. 2-It has been previously established that a transcription factor, even-skipped or eve, plays an essential role on the specification of motoneurons that project to dorsal muscles in Drosophila.
In order to understand how neurons are individually programmed, we have developed a novel approach that involves FACS isolation of small populations of genetically defined neurons and mRNAa profiling. We use the UAS/Gal4 system to express UAS-mCD8GFP specifically on the aCC, and RP2 Dorsal Motoneurons (dMNs) in fly embryos carrying the RN2Gal4 driver element. We perform single cell dissociation of embryos by trypsinization, sort GFP positive dMNs by FACS, and then we extract RNA from isolated cells.
We have isolated mRNA from dMNs from wild-type and eve mutant embryos, and performed microarray hybridization to identify eve-regulated genes. We then selected all genes that are differentially expressed with a false discovery rate of FDR less than 0.05 (5%).
In the overall comparison, we found 154 differentially expressed genes in eve mutant aCC and RP2 cells relative to wild type cells. Based on the biological process involved, we categorized these genes in following groups: Cell Signaling, Regulation of gene expression, Cell cytoskeleton associated, Cellular Transport, Extracellular matrix and Cell adhesion, and other enzymes.
26 gene were found to be specifically involved in three different neurological processes: axon grow and path finding, neurotransmitter biosynthesis and secretion or neuromuscular junction and synapse development.

ROLE OF THE NEUROTROPHIN RECEPTOR TRKB AND PRESYNAPTIC AXONAL SPROUTING IN HYPEREXCITABILITY AFTER INJURY.

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Posttraumatic epilepsy is a common and serious complication of acute traumatic brain injury. Seizures occur after a latent period of months to years. This delay in epileptic development suggests the initiation of a slow process after injury leading to a permanently epileptic brain. The delayed growth of new axon collaterals may be one such process. We have observed that transection of the Schaffer collateral (SC) pathway *in vitro* results in axonal sprouting by CA3 pyramidal cells and increases the probability that CA3 cells are connected by excitatory synapses. The extent of axonal sprouting is correlated with hyperexcitability, and transgenic mice with reduced trkB expression are less likely to exhibit axonal sprouting after SC transection. We now extend these findings using mice in which a single amino acid mutation in the trkB receptor (F616A) has been knocked-in, rendering it susceptible to blockade by the novel small molecule 1NMPP1 (Chen et al., 2005). $TrkB^{F616A}$ receptors are fully functional without drug and allow for full pharmacological blockade in the presence of the drug. SC pathway transection was performed in hippocampal slice cultures derived from $trkB^{F616A}$ mice at day 14 *in vitro*; cultures were treated with 1NMPP1 or normal media. Cultures were processed for immunohistochemical and Western blot (WB) analysis of GAP43, a marker for growing axons. To determine the contribution of trkB receptors and axonal sprouting under more physiological conditions, we performed SC transections *in vivo* in $trkB^{F616A}$ mice. Extracellular recordings of acute physiology slices were performed to determine hyperexcitability, and WB analysis performed to determine GAP43 levels after lesion. Our *in vitro* model revealed that the number of GAP43 immunoreactive fibers in the vicinity of the lesion was significantly reduced in 1NMPP1 treated cultures. Blockade of the trkB receptor with 1NMPP1 prevented the increases in GAP43 protein levels that were observed after the lesion. Extracellular recording in area CA3 from acute hippocampal slices obtained from the *in vivo* model showed a marked increase in their coastline bursting index indicating hyperexcitability. WB analysis of GAP43 levels indicated an increase GAP43 protein following the lesion as compared to sham controls. We confirm that lesion induced neurotrophin-trkB signaling is a critical promoter of axonal sprouting after injury. These data will provide a better understanding of the role of trkB receptor signaling and axonal sprouting after traumatic brain injury.

CELL SPECIFIC REGULATION OF SEMAPHORIN 3F SIGNALING MODULATES GABAERGIC CIRCUITRY

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Neurodevelopmental disorders such as autism result from GABAergic dysfunction involving complex multifactoral etiologies including gene-environment interactions. Molecular and genetic studies suggest that signaling pathways necessary for GABA interneuron development are disrupted in both Autism Spectrum Disorders (ASD) and epilepsy. Indirect genetic evidence suggests that one particular signaling pathway; the semaphorin (Sema) pathway is involved with both disorders (Gant et al, 2009). An interneuron dependent phenotype recapitulated in mice homozygous null for neuropilin 2 (NPN2), the receptor of Semaphorin 3F (Sema 3F), is similar to neuropathology found in ASD brain. Thus, mutants in neuropilin and semaphorin signaling are important mouse models for the understanding of neurodevelopmental disorders, especially those with GABAergic dysfunction.

The semaphorin signaling pathway mediates GABAergic interneuron migration and placement during neocortical development. Powerful new tools for genetic analysis were followed with classical molecular biological approaches to test the hypothesis that cell specific genetic variation in Semaphorin 3F (Sema 3F) signaling affects formation and function of GABAergic circuitry. These in silico analyses provided high confidence associations among mouse genomic loci and transcriptional regulatory proteins linked to GABAergic differentiation, PI3K/Akt signaling, and Sema 3F expression. Subsequent empirical analysis with mice homozygous null for Sema 3F in GABAergic neurons substantiated these predictions. Notably, deletion of Sema 3F in interneurons but not excitatory neurons during early development increase the number of interneurons, mRNAs for cell specific GABAergic markers, and increased GABAergic synaptic protein concentrations, accompanied by increased seizure susceptibility. Deletion of Sema 3F signaling in either cell type during development regulated the mTOR pathway, providing a plausible explanation for the change in interneuron survival in adult animals. In conclusion, Sema 3F expression in developing interneurons is necessary but not sufficient for proper development of GABAergic circuitry.

VEGFR2 (KDR/FLK1) SIGNALING MEDIATES AXON GROWTH IN RESPONSE TO SEMAPHORIN 3E IN THE DEVELOPING BRAIN

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Common factors are thought to control vascular and neuronal patterning. Here we report an in vivo requirement for the vascular endothelial growth factor receptor type 2 (VEGFR2) in axon tract formation in the mouse brain. We show that VEGFR2 is expressed by neurons of the subiculum and mediates axonal elongation in response to the semaphorin (Sema) family molecule, Sema3E. We further show that VEGFR2 associates with the PlexinD1/Neuropilin-1 (Nrp1) receptor complex for Sema3E and becomes tyrosine-phosphorylated upon Sema3E stimulation. In subicular neurons, Sema3E triggers VEGFR2-dependent activation of the phosphatidylinositol-3 kinase (PI3K)/Akt pathway that is required for the increase in axonal growth. These results implicate VEGFR2 in axonal wiring through a mechanism dependent on Sema3E and independent of vascular endothelial growth factor (VEGF) ligands. This mechanism provides an explanation as to how a semaphorin can activate an axon growth promoting response in developing neurons.

THE SECRETED TWO-IG DOMAIN PROTEINS ZIG-5 AND ZIG-8 REGULATE SAX-7/L1CAM TO MAINTAIN NERVOUS SYSTEM ARCHITECTURE

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Neuronal circuitries established during development must persist throughout life. Individual neurons need to maintain their interactions with their synaptic partners and their cellular environment. This is a challenge to the structural integrity of an embryonically patterned nervous system as an animal increases its size postnatally, remodels parts of its anatomy and incorporates new neurons. In addition, body movements, injury and ageing generate physical stress on the nervous system. Recent research in *C. elegans* has revealed that dedicated maintenance mechanisms ensure that precise neuronal positioning and organization of neuronal ensembles is maintained throughout the life of an animal[1].

Two types of molecules appear fundamental for the maintenance of the brain, the L1-like SAX-7 cell-adhesion and two-Ig domain protein ZIGs. L1CAM/SAX-7 is a cell adhesion molecule that is conserved in mammals. Congenital disorders in L1 lead to severe neurological defects, including mental retardation in humans[2], and depletion of L1 specifically in the brains of adult mice renders these mice unable to learn, highlighting the importance of L1 in the adult brain[3]. Although mutations in mice are available, the complexity of the mammalian nervous system has prevented a systematic analysis of their function. ZIGs are small proteins that contain two Ig domains and function to maintain the architecture of the nervous system in the nematode. Numerous similar proteins exist in mammals, many of which are expressed in the adult mouse brain, but their function has not been addressed.

Our studies demonstrate that L1 functions in neurons to maintain the organization of neuronal circuits in the worm, and that two central Ig domains within L1 are critical for this function. L1 exists as two distinct isoforms, a long and a short form, which differ in the number of extracellular Ig domains and in their adhesiveness[4,5]. Strikingly, the long form appears to be auto-inhibited by the folding of the two external Ig domains, giving rise to a closed L1 conformation. Our analyses indicate that the precise and local adhesiveness generated by L1 depends on ZIG-5 and ZIG-8. Based on our evidence, we hypothesize that ZIG-5 and ZIG-8 heterophilically interact with the external most Ig domains of L1, leading to an open L1 conformation and thus allowing the critical central Ig domains to mediate cell adhesion and thus the maintenance of normal neuronal projections and function.

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SYNAPTIC ADHESION BY SynCAM 1 DRIVES AND MAINTAINS EXCITATORY SYNAPSES IN THE DEVELOPING BRAIN AND REGULATES THEIR PLASTICITY

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The molecular mechanisms of synapse formation in the developing brain remain incompletely understood. Moreover, it is unclear to which extent proteins that can organize new synapses also act in the mature brain to modulate synaptic properties such as ultrastructure and plasticity. Aiming to address these questions, we have analyzed the synaptic cell adhesion molecule SynCAM 1 in overexpressing and knock-out mice. SynCAM 1 is an immunoglobulin superfamily member that mediates adhesive interactions across the synaptic cleft. Previous studies in cultured neurons have shown that SynCAM 1 induces presynaptic terminals and increases excitatory neurotransmission. To understand its functions *in vivo*, we generated transgenic mice that overexpress SynCAM 1 from a temporally regulated promoter in excitatory forebrain neurons. SynCAM 1 overexpressed in these mice is sorted properly to excitatory synapses as shown by immunohistochemistry and we find by co-immunoprecipitation that it interacts with its endogenous partners. Importantly, the elevated synaptic expression of SynCAM 1 increases excitatory synapse number *in vivo* as shown by electrophysiological recordings and morphological measurements. Conversely, the loss of SynCAM 1 in KO mice results in fewer synapses with altered ultrastructure. Our analysis of SynCAM 1 overexpression within select developmental stages reveals that SynCAM 1 acts first to promote functional excitatory synapses and is then required to maintain this increase in synapse number.
Endogenous SynCAM 1 is not only expresssed in early postnatal development but also in the adult brain. We therefore addressed whether SynCAM 1 modulates mature synapses. Interestingly, we find that SynCAM 1 expression regulates the plasticity of mature synapses as shown by electrophysiological slice recordings. This organization of synapses by SynCAM 1 in the adult brain affects spatial learning but not other behaviors. These reciprocal effects of SynCAM 1 increase and loss reveal that this synaptic adhesion molecule contributes to the regulation of synapse number and plasticity, and impacts how neuronal networks undergo activity-dependent changes.

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COINCIDENCE DETECTION OF EPHRIN-A AND GDNF SIGNALS IN MOTOR AXONS

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During development axons are concomitantly exposed to multiple signals that guide their trajectory to the final targets. Stimuli that would individually trigger opposite cellular effects (e.g., attraction or repulsion) become integrated to produce a net signaling output that translates into stereotypical navigational decisions. We are investigating how guidance signals are integrated in motor axons that make subtype-specific decisions at the base of the limb to innervate either dorsal or ventral muscles. As this binary topographic projection map is under the control of multiple signaling pathways (including ephrins, semaphorins, and growth factors) we reason that coincident detection mechanisms might be in place to expand the range of responsiveness of navigating growth cones through the combinatorial use of a limited number of guidance components.

Motor axons select a dorsal trajectory in the limb using an unconventional growth-promoting GDNF pathway, mediated by the kinase receptor c-RET, in combination with ephrin-A:EphA ('forward') repulsive signaling, which relies on the kinase activity of EphAs. The same class of neurons expresses ephrin-As, which stimulate axon growth upon engagement with cognate EphAs. This 'reverse' signaling mode via glycosyl-phosphatidyl-inositol (GPI)-anchored ephrin-As, attached to the external surface of the plasma membrane, involves transmembrane 'co-receptors'.

We propose a model for coincident detection of ephrin-A and gdnf signals whereby gdnf binding to GPI-anchored GFRalpha1 induces the translocation of c-RET into ephrin-rich membrane microdomains enabling c-RET to act as a co-receptor for EphA:ephrin-A 'reverse' signaling to promote axon growth.

WNT SIGNALLING PROMOTES DENDRITIC SPINE GROWTH AND SYNAPTIC STRENGTH THROUGH CAMKII

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The balance between excitatory and inhibitory synapses is crucial for normal brain function. Wnt proteins stimulate synapse formation by increasing synaptic assembly. However, it is unclear whether Wnt signalling differentially regulates the formation of excitatory and inhibitory synapses. Here we demonstrate that Wnt7a preferentially stimulates excitatory synapse formation. In cultured hippocampal neurons, Wnt7a increases the number and co-localisation of excitatory pre- and postsynaptic markers, whereas inhibitory synapses are unaffected. Wnt7a or postsynaptic expression of Dishevelled-1 increases the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs), but not miniature inhibitory postsynaptic currents (mIPSCs). Consistently, Wnt7a signalling increases the density and maturity of dendritic spines, whereas mutant mice deficient in Wnt signalling exhibit defects in spine morphogenesis *in vivo*. Expression of PSD-95-Vim-CFP, a synaptic reporter of Ca2+/Calmodulin-dependent protein kinase II (CaMKII) activity, demonstrates that Wnt7a rapidly increases CaMKII activation in spines. Importantly, CaMKII inhibition abolishes Wnt7a-mediated spine growth and increased synaptic strength. Our findings identify a novel mechanism by which Wnts selectively promote excitatory synaptogenesis through CaMKII-dependent modulation of dendritic spine morphology.

IDENTIFYING NOVEL DOWNSTREAM EFFECTORS OF NKX2.8/9 THAT FACILITATE SACMN AXON EXIT FROM THE SPINAL CORD

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Coordinated motor behaviors rely on the proper targeting of motor neuron (MN) axons to their appropriate body muscles. En route to their synaptic targets, embryonic spinal motor axons must make multiple pathfinding decisions, including when and where to exit the spinal cord. The molecular mechanisms that facilitate the projection of MN axons out of the spinal cord through ventral or dorsal exit points remain obscure. Whereas the majority of spinal MN axons leave the CNS through ventral exit points, spinal accessory motor neurons (SACMNs) project dorsally directed axons toward and through the lateral exit point (LEP) and assemble into the spinal accessory nerve (SAN), which innervates neck and back muscles. We previously showed that a monoclonal antibody specific for BEN, an Ig domain-containing cell surface protein, selectively labels SACMN cell bodies and their axons. Given that SACMN and their axons are readily identifiable and that SACMN are the sole MNs whose axons leave the CNS through the LEP, these particular motor axons represent a model system for investigating the mechanisms underlying motor axon exit. Moreover, we previously showed that SACMN axons fail to exit the mouse spinal cord in the absence of Nkx2.8/9, a homeodomain transcription factor (TF), while the pathfinding of ventral-exiting MNs remains unperturbed. Further analyses of *Nkx2.8/9* deficient mice revealed that SACMN axons form an ectopic nerve that projects longitudinally *within* the spinal cord proper. Although there is increasing evidence that TFs, including Nkx2.8/9, control discrete aspects of axon guidance, the identities of the corresponding downstream effector genes are largely unknown across species. To identify novel downstream targets of Nkx2.8/9, we performed a microarray screen designed to retrieve genes that are differentially expressed in *Nkx2.8/9* deficient versus wild type mice. From this screen, we identified Hox genes, Robo2 and members of the Slitrk family; novel transmembrane proteins with significant homology to Slit and Trk proteins whose functional role during development remains to be elucidated, that are each likely to be up-/down-regulated in *Nkx2.8/9* null mouse embryos. Consistent with the possibility that Robo2 is a putative downstream effector of Nkx2.8/9, most SACMN axons also fail to exit the spinal cord in mice lacking Robo2. In addition, analyses of Slit lacking mice raises the possibility that Robo2 regulates SACMN axon exit via a novel Slit-independent mechanism. Ongoing studies are directed toward determining whether Nkx2.8/9 directly regulates Robo2. These findings provide novel insights into the molecular control of MN axon exit by linking Nkx2.8/9 to the regulation of Hox genes and Robo/Slitrk cell surface proteins.

THE RECEPTOR TYROSINE PHOSPHATASE *CLR-1* IS REQUIRED FOR *C. ELEGANS* AXON REGENERATION

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Axons of the adult mammalian brain and spinal cord do not regenerate after injury. However, there is substantial axon growth after peripheral nerve injury, even in humans. Recent expression studies in rodents have identified dozens of genes that are upregulated after axotomy with or without successful axon regeneration. Unfortunately, no functional role is yet defined for most of these genes, and the task of assessing function one-by-one *in vivo* is dauntingly slow and technically challenging in the mouse. *C. elegans* is a useful model for studying the genetics of axon regeneration. Individual GFP-labeled axons can be lesioned by laser microsurgery, and their regrowth can be monitored *in vivo*. *C. elegans* motor neurons display robust regeneration and can reestablish functional connections after laser axotomy. Genes replicated in multiple studies as differentially expressed in microarray or RT-PCR analyses of mammalian CNS or PNS regeneration were compiled from the literature (1-3) and from preliminary studies in our laboratory. Of the top 50 genes, 30 genes had *C. elegans* homologues and 25 of these had known and viable *C. elegans* loss of function alleles. We screened each of these candidate genes for a contribution to regeneration in the *C. elegans* GABA motor neurons. We identified *clr-1*, which encodes a receptor tyrosine phosphatase related to the LAR family, as a modulator of regeneration. *clr-1* loss of function mutants are capable of regeneration per se, as the percentage of fibers that initiate growth after axotomy in *clr-1* mutants is similar to that seen in wild-type worms. However, *clr-1* mutant regenerating axons display extensive lateral branching that prevents long distance growth. Rarely do *clr-1* axons successfully cross the lesion site to reach the dorsal cord. These data may parallel recent studies on PTP-sigma and other LAR family members in mammals(4), and highlight a role for receptor tyrosine phosphatases in axonal regeneration.

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EXPRESSION OF DSCAM AND SIDEKICK PROTEINS AT THE DEVELOPING MOUSE OPTIC CHIASM

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The optic chiasm is an important midline choice point where retinal ganglion cell (RGC) axons from each eye diverge to targets on both sides of the brain, setting up binocular vision. While several cues essential for guidance at the optic chiasm have been identified, it is clear other signals are required.

We have begun to investigate the role of the highly related homophilic cell adhesion molecules, Down's syndrome cell adhesion molecule (Dscam), Dscam Like1 (DscamL1), Sidekick1 (Sdk1) and Sidekick2 (Sdk2) in directing RGC axon guidance. Dscam is a key regulator of midline axon guidance in Drosophila and all 4 proteins are essential for the normal wiring of the vertebrate retina.

Using in situ hybridisation we have examined the expression patterns of these genes in E12.5-E17.5 mouse embryos, the period when the optic chiasm is developing. At all ages *Dscam* and *Sdk1* are expressed strongly in the RGC layer of the retina whereas *Sdk2* is more widely distributed throughout the retina. *Dscam* and *Sdk2* also are expressed in the region of the developing optic chiasm. Expression of *Dscam* borders the optic pathway whereas *Sdk2* is expressed by the glia of the optic nerve and midline. The significance of these expression patterns is being investigated *in vivo* and *in vitro*. We are currently investigating the functional significance of Dscam *in vivo* using Dscam KO mice. Our initial analyses of these mice have demonstrated that Dscam plays an important role in controlling retinal ganglion cell number and retinal organisation postnatally. *In vitro* work has also shown that Dscam is capable of enhancing axonal outgrowth from embryonic retinal explants in culture conditions.

STRUCTURE AND FUNCTION OF THE INTRACELLULAR REGION OF THE PLEXIN-B1 TRANSMEMBRANE RECEPTOR

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Plexins are transmembrane receptors for semaphorin ligands that regulate cell migration processes in several setting, including axon guidance in the developing nervous and angiogenesis in the cardiovascular system. The receptor's anti-migratory/cell collapse function can be dysregulated by oncogenic mutations[1], which are known to contribute to cancer metastasis. Upon activation, plexin initiates signaling events that involve several small GTPases of the Ras and Rho families (R-Ras, Rac1, Rnd1 and RhoD) and that regulate cytoskeletal dynamics and cell adhesion. Plexins are unique amongst transmembrane receptors because their intracellular domains interact directly with the small GTPases. Specifically, plexins include domains with homology to GTPase activating proteins (GAPs) and these are thought to possess GAP activity toward R- and M-Ras. The structure of the RhoGTPase binding domain (RBD) [2] and a recent determination of the x-ray structure of the entire intracellular region of plexin-B1 shed new light on the function and substantiate a possible mechanism for activation [3]. We show that the receptor functions via a conformational shift in a monomer-multimer equilibrium on the intracellular side. Implications for the different roles of Rnd1 and Rac1 in receptor activation are discussed.

Selected papers from the Buck lab. on plexin structure and function:
[1] Structure 16, 246-258 (2008) [2] J. Biol. Chem. 282, 37215-24 (2007)
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LIMITATION OF ADULT CNS AXONAL GROWTH BY THE NOGO/NGR1 PATHWAY

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Functional recovery after trauma to the adult brain or spinal cord is limited in part by the CNS myelin proteins, Nogo-A, MAG, and OMgp. All three are produced by oligodendrocytes and share neuronal receptor mechanisms through Nogo Receptor (NgR1) and PirB.
In recent work we compared mice singly, doubly or triply mutant for these 3 myelin ligands (1). Both *in vitro* outgrowth assays and *in vivo* spinal cord injury studies revealed a hierarchy of activity. Loss of Nogo-A allows corticospinal (CST) and raphespinal axon growth above and below the injury, as well as greater behavioral recovery than in wild type or heterozygous mutant mice. In contrast, deletion of MAG and OMgp stimulates neither axonal growth nor enhanced locomotion. Nogo-A/MAG/OMgp triple mutant mice exhibit greater axonal growth and improved locomotion, consistent with a principal role for Nogo-A and synergistic actions for MAG and OMgp, presumably through shared receptors. A parallel study of these 3 ligands observed reduced *in vitro* axon inhibition after Nogo deletion, but no synergy with MAG or OMgp (2). Injury studies detected variable degrees of injury-induced *in vivo* axon sprouting in single mutants, but no synergy and no frank regenerative growth (2). Of note, these two triple knockout studies utilized different Nogo alleles. As single mutants, different Nogo alleles have documented differences in the extent of both axon sprouting and regeneration *in vivo* (3, 4). Here, we have investigated the mechanism whereby nogoab$^{trap/trap}$ mice differ from nogo-ab$^{atg/atg}$ mice. With the atg allele mice, there is compensatory up-regulation of many oligodendrocyte genes. In mice with the trap allele, the persistently expressed N-terminal 309 amino acid Nogo-A fragment traffics to the nucleus to prevent the gene compensation seen with the atg mice. Thus, the trap line allows a more accurate assessment of Nogo-A's role without chronic compensation. This re-emphasizes the caution required for interpreting negative results from a loss-of-function study.

Most SCI studies use CST tracing as the principle measure of axon regeneration, but this requires extrinsic markers that label at best ~5% of axons. We have developed a BAC transgenic GFP marker that labels ~100% of CST fibers in the spinal cord. With this high fidelity marker, we have re-examined CST regeneration in NgR1(310)ecto-Fc treated and NgR1 null mice and document regenerative growth of axons after SCI.

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CONTROL OF AXONAL TILING IN THE *DROSOPHILA* VISUAL SYSTEM

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Axonal/dendritic tiling is an important mechanism that patterns neuronal circuitry in the developing nervous system. Tiling refers to the avoidance between neurites from certain same-type neurons for complete but non-overlapping coverage of receptive fields, which is essential for spatial discrimination of sensory information. We have previously identified Turtle, a member of the conserved Turtle/DASM1/IgSF9 subfamily of the immunoglobulin superfamily as a key mediator of the projection of the R7 photoreceptor axon in *Drosophila*. Turtle mediates R7 tiling in a homophilic manner. To further understand the mechanism of Turtle action in axonal tiling, we have taken a combination of molecular and genetic approaches to identify Turtle-interacting proteins. We found that Baby Turtle (Btutl), another member of Ig-superfamily proteins, interacts with Turtle both biochemically and in cultured Drosophila S2 cells. Immunohistochemical analysis showed that Btutl is present at R7 terminal layer in the medulla. Furthermore, genetic analysis revealed an interaction between *btutl* and *turtle* in mediating R7 axon tiling. Our results are consistent with a model in which Turtle antagonizes the function of Btutl to mediate R7 axon tiling.

FLOOR PLATE-DERIVED NRCAM AND GDNF COOPERATE TO CONTROL PLEXIN-A1 LEVEL AND RESPONSIVENESS TO SEMAPHORIN3B DURING COMMISSURAL AXON GUIDANCE

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Extensive studies showed that the formation of commissural pathways requires highly precise control of axon sensitivity to multiple midline-derived guidance cues, axons acquiring responses to local repellents upon crossing, thus becoming instructed to move away. In recent work, we described a molecular mechanism in vertebrates controlling the gain of response to a midline repellent, Semaphorin3B (Sema3B) (Nawabi et al, Genes&Dev, 2010). We provided evidence that a Sema3B-Plexin-A1 signaling is required to guide spinal commissural axons across the floor plate (FP) in which they cross the midline. At pre-crossing stage, growth cone expression of the Sema3B signaling coreceptor Plexin-A1 is prevented by endogenous calpain1-mediated processing, resulting in silencing of growth cone responsiveness to Sema3B. During FP crossing, this cleavage is suppressed by local FP signals, allowing Plexin-A1 accumulation in the growth cone and sensitization to Sema3B. Here, we investigated the nature of the FP signals using mouse models, in vitro cultures and biochemical approaches. We provide evidence for a cooperative role of the Ig Superfamily Cell Adhesion Molecule NrCAM, and the Neurotrophic Factor gdnf. In vitro, NrCAM and gdnf could trigger Plexin-A1 up-regulation and sensitization of commissural growth cones to Sema3B. In vivo, NrCAM and gdnf genetic deletion differently affected commissural axon guidance. NrCAM deficiency induced stalling in the FP, while gdnf deficiency induced rostro-caudal guidance errors, both defects being reminiscent of those observed in mouse embryos lacking Sema3B and Plexin-A1. The generation and analysis of double NrCAM/gdnf homozygotes and heterozygotes confirmed that NrCAM and gdnf act in synergy to regulate Plexin-A1 level and responsiveness to Sema3B. Interestingly, biochemical analysis of commissural tissues stimulated ex vivo by NrCAM and gdnf showed that these two signals up-regulate different Plexin-A1 forms, which may thus have distinct functional properties. These data provide a molecular basis for understanding the specific roles of NrCAM and gdnf in the Sema3B/Plexin-A1-mediated signaling during commissural axon guidance and the different phenotypes resulting from their deletion.

MUD IS REQUIRED FOR AXON GUIDANCE AT THE MIDLINE OF THE *DROSOPHILA* CENTRAL NERVOUS SYSTEM.

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The study of axon guidance at the midline of the central nervous system in the *Drosophila* embryo has allowed the identification and study of factors directing axonal growth. The initial simple model of a balance of the Netrin attractant and Slit repellent guiding axons at the midline has recently become complicated by the discovery of additional midline guidance pathways and mechanisms, involving *robo2*, *Dscam* and *turtle*. We have identified the cytoplasmic coiled-coil protein Mushroom Body Defect (mud) as a further factor whose activity to guide commissural axons is revealed in the absence of Netrin signalling. *mud* is expressed throughout the ventral nerve cord when axons extend and becomes restricted to a subset of glia by the end of neurogenesis. The protein is expressed in an intriguing subcellular pattern in neurons and we show that its role in axon guidance is independent of its previously described function in neuroblast polarity. Mud has an additional role to direct the outgrowth of a subset of longitudinal axons. Genetic interaction studies show that mud is acting downstream of some, but not all, of the pathways known to be required for midline guidance. We are using cell and molecular biological methods to further elucidate the function of mud in axon guidance and position it within the identified pathways.

ROBO-MEDIATED REPULSION IN AXON GUIDANCE: PROTEOLYTIC REGULATION OF RECEPTOR ACTIVITY

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Understanding how an individual growth cone deploys its guidance receptors to make guidance choices is critical to learning how proper wiring is established in development. Roundabout (Robo) is one such guidance receptor that mediates repulsion from its ligand Slit in both humans and our model organism, *Drosophila*. As with other guidance receptors, Robo influences pathfinding by modulating intracellular cytoskeletal dynamics; how its activity on the cytoskeleton leads to repulsion from the midline is not completely understood. Here we present preliminary genetic and biochemical evidence supporting a role for proteolytic processing in regulating Slit-Robo repulsive signaling. We find that that Kuz-mediated cleavage positively regulates (1) repulsion, (2) exclusion of Robo from the commissures, and (3) an overall reduction in Robo protein levels. We also present preliminary data that (1) show that Robo cleavage by gamma-secretase can occur *in vitro* and (2) suggest that gamma-secretase negatively regulates repulsive signaling *in vivo*. Taken together, our data suggest that proteolytic processing provides a mechanism for the precise temporal control over the activity of the Robo receptor.

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PLASTICITY IN THE BRAINSTEM RESPIRATORY NETWORK OF THE *LOOPTAIL* NEURONAL MIGRATION MUTANT MOUSE

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The effects of aberrant neuronal connectivity on physiology and behavior are well established. However, less is known about the effects of aberrant neuronal position on these processes, especially in mammals. Therefore, we investigated the formation and properties of brainstem neurons that generate respiratory rhythm in *Looptail* (*Lp*) mutant mice, which are loss-of-function for Vangl2, a transmembrane component of the Wnt/PCP pathway, and where the facial motor nucleus is mislocated due to a failure of tangential neuronal migration. Using molecular markers and calcium imaging of hindbrain explants, we found that neurons of the embryonic parafacial (e-pF) respiratory oscillator could be detected in *Looptail* heterozygotes and homozygotes, even though the facial motor neurons failed to move caudally out of rhombomere 4 (r4) into r6. Whereas e-pF cells are normally found in close apposition and ventrolateral to the facial motor nucleus in r6, most e-pF neurons in *Looptail* mutants were found in aberrant locations, medially within r5/r6 (*Lp*+/-) or r4 (*Lp*-/-). Nevertheless, pharmacological analyses showed that these misplaced e-pF neurons exhibited characteristic cellular and network properties. Furthermore, using hindbrain slices, we found that the second respiratory oscillator, the preBötzinger complex, developed normally in *Looptail* mutants. These results suggest that the Wnt/PCP pathway regulates the migration of e-pF neurons, a critical respiratory pacemaker. Surprisingly, functional respiratory oscillators are still established in *Looptail* mutants, highlighting the plasticity of the neuronal network mediating an essential physiological function.

THE WNT/PCP PROTEIN VANGL2 FUNCTIONS IN THE FLOOR PLATE TO REGULATE FACIAL MOTOR NEURON MIGRATION IN ZEBRAFISH

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The transmembrane protein Van gogh-like 2 (Vangl2) is a component of the non-canonical Wnt/Planar Cell Polarity (PCP) signaling pathway, and is required for tangential migration of facial branchiomotor neurons (FBMNs) from rhombomere 4 (r4) to r5-r7 in the vertebrate hindbrain. In zebrafish, *vangl2* is ubiquitously expressed in neural and non-neural tissues during motor neuron migration, and functions non-cell autonomously. To determine the identity of the cell type(s) in which *vangl2* functions, we performed targeted transplantation and knockdown experiments to address the role of endodermal, mesodermal, and neural tissues in FBMN migration. Elimination of *vangl2* in mesendoderm through targeted transplantation, or of endoderm entirely did not perturb FBMN migration, suggesting that *vangl2* expression in non-neural tissues is not necessary for this process. Interestingly, in some transplant experiments, the presence of *vangl2*-deficient cells in the floor plate and ventral neuroepithelium adjacent to FBMNs inhibited their migration.

To directly test a role for *vangl2* in floor plate cells, we performed targeted cell transplantation to manipulate the genotype of the floor plate. In wild-type host embryos containing *vangl2*-deficient floor plate cells in r4, FBMN migration was frequently blocked. Importantly, some *vangl2*-deficient FBMNs migrated out of r4 in the presence of wild-type floor plate cells. These data suggest strongly that *vangl2* function is required primarily in the floor plate to support FBMN migration in zebrafish. Consistent with this, FBMNs failed to migrate in mouse mutants lacking the floor plate. These results identify a novel role for floor plate cells in regulating directed neuronal migration mediated by the Wnt/PCP signaling pathway.

ANALYSIS OF BRAIN DEVELOPMENT IN SLIT TRIPLE KNOCKOUTS

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In vertebrates, three Slits (Slit1-Slit3) secreted by floor plate cells cooperate to prevent commissural axons from recrossing the midline. In mice, there are only minor axon guidance defects in Slit single knockouts due to the overlapping expression patterns of the Slits in the developing brain. In the spinal cord, commissural axons are misguided only when all Slits are simultaneously deleted (Long et al., 2004). However, survival is reduced in the *Slit3* KO due to heart defects, and the *Slit2* KO dies shortly after birth. Therefore, the function of Slits in brain development, in particular in postnatal life is largely unexplored.
To overcome this problem, we generated *Slit2* conditional knockout mice (*Slit2*^{lox}). These mice were crossed with *Slit1* and *Slit3* knockouts to generate all combinations of *Slit* double and triple knockouts. *Slit1;Slit2*^{lox}*;Slit3* are viable and fertile and the anatomical organization of their brain appears similar to wild type mice. To delete Slits in specific cells and at specific time points, we crossed Slit2lox mice and compounds *Slit* KO with various transgenics expressing Cre recombinase.
To delete Slits in the floor plate we generated a line expressing Cre under an Hoxa1 enhancer (Li and Lufkin, 2000; Hoxa1-cre). Cre was highly expressed in the floor plate throughout the spinal cord and in the caudal hindbrain. Slit2 deletion was confirmed using an exon 8 specific probe. We also confirmed that all Slits were absent in *Slit1;hoxa1-cre:Slit2*^{lox}*;Slit3* by in situ hybridization and by performing binding with a Robo-AP fusion protein. Interestingly, these mice are viable but strongly ataxic. Using DiI tracing we found that in E12.5 *Slit1;hoxa1-cre:Slit2*^{lox}*;Slit3* mutants, commissural axons wander or stall at the midline, as previously described in constitutive *Slit* triple KO.
The strategy was validated further by using FoxG1-cre transgenics which drive cre expression in the telencephalon. *Slit1;FoxG1:cre;Slit2*^{lox} mice were viable and did not have any obvious behavioral deficits. However, many axonal tracts were highly disorganized: the corpus callosum was interrupted and an additional commissure was observed in addition to the anterior commissure. These defects are similar to what has been described in embryos from *Slit1;Slit2* double KO mice (Plump et al., 2002).
We are know conducting a detailed phenotypic analysis of brain defects in these mice.

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INDUCTION OF GLYCINERGIC NEUROTRANSMISSION IN CENTRAL NEURONS

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Neuronal transmitter phenotype is determined mainly by intrinsic transcription factors during development. Recent studies suggest that extrinsic factors may also play a role but the underlying molecular mechanism remains largely unknown. Here, we report an inducible plasticity of inhibitory neurotransmitter phenotype after altering postsynaptic receptors together with cell adhesion molecules. In embryonic hypothalamic and hippocampal cultures, inhibitory neurotransmission is mediated by GABAergic neurons only, although most neurons do express high level of glycine receptors (GlyRs) in addition to GABAA-Rs. Despite the lack of glycinergic events in pure neuronal cultures, functional glycinergic synapses can be induced in cocultured HEK 293T cells expressing GlyRs and a cell adhesion molecule neuroligin-2 (NL-2). Ectopic expression of NL-2 or GlyRs alone in central neurons cannot change GABAergic transmitter phenotype. However, coexpression of NL-2 and GlyRs in central neurons induces robust functional glycinergic synapses. Immunostaining revealed that glycine is present in the nerve terminals of central neurons but glycine transporter GlyT2 is not. Our data demonstrate that central neurons have presynaptic glycine as well as postsynaptic glycine receptors, but lack normal glycinergic neurotransmission. Molecular manipulation of postsynaptic components can induce glycinergic neurotransmission in central neurons, suggesting a novel type of synaptic plasticity.
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RETINAL INPUT INSTRUCTS AFFERENT CONNECTION REFINEMENT OF VISUAL CIRCUITRY

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Mature visual system contains precise afferent connections across multiple relay stations in the brain. Visual circuit relays information from retina, through lateral geniculate nucleus (LGN), to primary visual cortex (V1) where layer5 cortical neurons extend axons out of the cortex to target various subcortical regions. During development, layer5 visual cortical neurons project axons to the spinal cord before undergoing stereotyped long-distance pruning with terminal arbors retained in the pontine nuclei. Thus,stereotyped pruning of the corticospinal connection is a milestone in the completion of visual circuit refinement. Here, we find that corticospinal axon pruning and corticopontine arbor refinement are carried out at the third postnatal week and completed around P21. The emergence of precise visual cortical neuron projection is influenced by retinal input. During the first two postnatal weeks, blockage of the spontaneous activity but not visually evoked-activity in the retina results in unpruned corticospinal axons and broad corticopontine termination zone. Previous studies find that the emergence of precise afferent connections in the LGN and V1 also depends on the retinal input before eye-opening (P12-P14). Taken together, retinal input instructs afferent connection refinement of the retino-geniculo-cortical pathway and precise visual circuitry emerges before the onset of critical period for cortical plasticity.

A NOVEL DROSOPHILA ZINC FINGER PROTEIN IS REQUIRED FOR GUIDING MOTOR AXONS AND MAINTAINING SYNAPTIC STABILITY AT THE NEUROMUSCULAR JUNCTIONS

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Synapse disassembly, and synapse formation, are both essential for precise wiring of neural circuitry. However, the molecular mechanism of synaptic disassembly remains poorly understood. Here we use the Drosophila neuromuscular junctions to perform a genome-wide forward genetic screen for mutants that disrupt the stability of NMJs. We identified a novel zinc finger protein as an important regulator of synaptic stability. Mutations of the zinc finger protein cause synapse to disassemble and retract. Members of zinc finger proteins are known to bind DNA directly and constitute the largest family of transcription regulators. The novel zinc finger protein contains three C2H2-type zinc-finger motifs, is expressed in a subset of motorneurons, and is localized in the nucleus, consistent with its potential role as a transcription regulator. We generate null alleles of the novel zinc finger protein and demonstrate that it is required presynaptically to maintain synaptic stability. In addition, mutants of this novel zinc-finger protein display significant axon guidance defects at muscle 12/13. Null mutants of the novel zinc finger protein are semi-lethal, with a few adult escapers. The surviving adult escapers are short-lived and have severe locomotor defects. Taken together, these findings suggest that the novel zinc finger protein functions in motorneurons in a cell-autonomous manner, and is required for development of NMJs at several stages. It is essential to reach correct targets at an early stage, and to maintain synaptic stability at a later stage.

DO ORTHOLOGS OF THE YEAST RAM PATHWAY MEDIATE WNT SIGNALING IN NEURONAL POLARITY?

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Wnts regulate cell migration, axon guidance and polarity along the *C. elegans* A/P axis[1]. For example, Wnts control the polarity of the mechanosensory neuron ALM[2,3]. Although single *cwn-1*, *cwn-2* or *egl-20* Wnt mutants display normal ALM polarity, the polarity of the ALMs is often reversed in *cwn-1; cwn-2* or *cwn-1; egl-20* double mutants. We find that ALM polarity also requires the Frizzled receptor MOM-5 and atypical Wnt receptor CAM-1. While Frizzled proteins mediate the effects of Wnts in many developmental contexts, how these molecules signal to control neuronal polarity is unclear. We find that the MIG-15 kinase and potential components of a MIG-15 signaling pathway might be novel Wnt effectors in neuronal polarity.

MIG-15, the *C. elegans* ortholog of Nck-interacting kinase (NIK) in mice and Misshapen in *Drosophila*, was shown to function in cell migrations that also require Wnt function[4,5,6]. *mig-15* mutants exhibit a low frequency of ALM polarity defects that was enhanced by a mutation in *cwn-1*, suggesting that MIG-15 could mediate the effects of Wnts in ALM polarity. In *S. cerevisiae*, Kic1p, a distant relative of MIG-15, acts in the RAM (regulator of Ace2p activity and cellular morphogenesis) signaling pathway to regulate polarized cell growth[7]. We asked whether *C. elegans* orthologs of RAM signaling molecules also regulate ALM polarity. Through RNAi and mutant analysis in a *cwn-1* sensitized background, we identified *mop-25.2* and *sax-2* as regulators of ALM polarity. The gene *mop-25.2* is the ortholog of yeast Hym1p, which physically interacts with Kic1p, and SAX-2 is the ortholog of the RAM scaffold protein Tao3p. SAX-2 acts in the ALM to establish its polarity. We were surprised to find that *cwn-1; sax-1* mutants did not have an ALM polarity phenotype. SAX-1 is the ortholog of the NDR kinase Cbk1p and a target of the Kic1p kinase. We are currently testing whether the other *C. elegans* NDR kinase WTS-1 provides an overlapping function in ALM polarity in the absence of SAX-1. We are now testing whether the RAM pathway homologs act in the Wnt pathway in ALM polarity.

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MEPSPS REGULATE THE GROWTH OF *DROSOPHILA* SYNAPSES

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Two forms of neurotransmission are found at excitatory chemical synapses, evoked Excitatory Post-Synaptic Potentials (EPSPs) which transfer information across the synaptic cleft by the release of multiple synaptic vesicles in response to an action potential and miniature Excitatory Post-Synaptic Potentials (mEPSPs) induced by the spontaneous release of single synaptic vesicles. Recent studies have found differences in the synaptic vesicle pools utilized during evoked and spontaneous release and shown that spontaneous release can be regulated by presynaptic calcium. These studies suggest that mEPSPs may have distinct physiological characteristics to EPSPs. However, while EPSPs are incontrovertibly essential to brain function, the *in vivo* role of mEPSPs remains unclear.

We find that mEPSPs regulate the growth and development of *Drosophila* glutamatergic neuromuscular junction (NMJ) synapses. We show that disruption of mEPSPs by either blocking vesicular glutamate release from the presynapse, or disrupting activation of glutamate receptors in the postsynapse inhibits synaptic terminal growth. In contrast, specifically inhibiting EPSPs but not mEPSPs does not affect synapse development. We further show that genetic manipulations to increase mEPSPs induce synaptic terminal expansion and we demonstrate that this overgrowth proportionally depends upon the level of mEPSPs. Therefore, mEPSPs can bidirectionally modulate synaptic terminal growth.

Our results show that mEPSPs function as a novel instructive trans-synaptic signal with unique properties in the regulation of synaptic structural development.

ANALYSIS OF AXON GUIDANCE PHENOTYPES IN RYK KNOCKOUT MICE

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The axon guidance receptor Ryk is expressed on cortical axons during development of the mouse corpus callosum, the major interhemispheric forebrain commissure. Guidance of callosal axons is dependent on both physical cues and morphogens. Our lab has shown that Wnt5a-Ryk interactions are responsible for the chemorepulsive guidance of postcrossing callosal axons away from the midline and into the contralateral hemisphere (Keeble et al., 2006).

In 25% of *Ryk*$^{-/-}$ mice on a mixed C57Bl/6J x 129/Sv background (*Ryk*$^{-/-mix}$) callosal axons successfully cross the midline but fail to project into the contralateral hemisphere, instead forming contralateral axon bundles. We have also examined the callosal phenotype in *Ryk*$^{-/-}$ mice on a pure 129/Sv background (*Ryk*$^{-/-129}$) using immunohistochemistry, lipophillic carbocyanine dye tract tracing and diffusion tensor magnetic resonance imaging (DTI). As seen in *Ryk*$^{-/-mix}$ embryos, we observe contralateral axon bundles in 11% of *Ryk*$^{-/-129}$ embryos. In addition, 78% of *Ryk*$^{-/-129}$ embryos display ipsilateral callosal axon bundles. DTI analysis has further revealed that other axon tracts including the fornix and internal capsule are also disrupted in *Ryk*$^{-/-129}$ embryos. We are currently verifying these defects by immunohistochemistry and tract tracing.

The increase in severity of callosal axon guidance defects in *Ryk*$^{-/-129}$ embryos compared to *Ryk*$^{-/-mix}$ embryos suggests that there may be differences in the signalling pathways activated by Wnt5a-Ryk in these mouse strains. We are currently investigating these signalling pathways in cultured cortical neurons from *Ryk*$^{+/+}$ and *Ryk*$^{-/-}$ embryos on both backgrounds. Our recent findings indicate that there is no change in intracellular β-catenin levels in *Ryk*$^{+/+mix}$ or *Ryk*$^{-/-mix}$ neurons cultured in the presence or absence of Wnt5a. This suggests that the canonical β-catenin-dependent Wnt signalling pathway is not activated by Wnt5a in cortical neurons.

Keeble TR, Halford MM, Seaman C, Kee N, Macheda M, Anderson RB, Stacker SA, Cooper HM (2006) The Wnt receptor Ryk is required for Wnt5a-mediated axon guidance on the contralateral side of the corpus callosum. J Neurosci 26(21):5840-5848.

THE PATTERN OF GLOMERULAR MAP FORMATION DEFINES RESPONSIVENESS TO AVERSIVE ODORANTS IN MICE.

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In many species, the detection and recognition of odors is critical to regulate behaviors essential for survival such as food foraging and avoidance of predators. The formation of complex stereotypic connections between olfactory sensory neurons (OSNs) and second order neurons in the olfactory bulb (OB) is believed to be important for accurate odorant information processing. In mice, ablation of OSNs that innervate the dorsal region of the OB leads to a loss of avoidance behavior in response to aversive and predator odorants[1]. It remains to be determined whether the accurate formation of a glomerular map in this region of the OB is required for these innate responses. Here, we have generated mice that lack expression of the axon guidance receptor Robo-2 in OSNs and found that ablation of Robo-2 expression leads to mistargeting of subsets of OSN axons within the dorsal region of the OB. Furthermore, these mice show decreased avoidance behavior toward the predator odorant trimethyl-thiazoline (TMT). Our results indicate that the pattern of glomerular innervation in the OB is critical for innate behavioral responses in mice.

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MOLECULAR DIVERSITY AND SOMATOTOPIC ORGANIZATION OF RUBRO-SPINAL PROJECTION NEURONS.

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Spinally-directed motor behaviors depend critically on the specificity with which supraspinal descending pathways activate motor neurons. Cerebral cortical and midbrain motor centers have been implicated in the control of voluntary motor output, but the organization, molecular identity, and intraspinal circuitry of these regulatory systems remains unclear. Descending projections from the red nucleus (RN) in the midbrain are thought to have a specialized role in fine motor control in mammals, prompting us to combine genetic and anatomical approaches to examine the organization of this pathway in mice. Injection of Cholera Toxin B retrograde tracer into the intermediate region of p7 spinal cord, at different rostrocaudal levels, revealed a topographic organization of projection neurons within the caudal RN -- dorsomedial neurons project to cervical spinal levels whereas ventrolateral neurons project to lumbar spinal cord. In addition, we detected a distinct rostral neuronal group with projections to all spinal levels.

To define molecular correlates of this somatotopy we used laser capture to isolate distinct subpopulations of red nucleus neurons for microarray analysis. We found that all rubrospinal neurons express the POU transcription factor Brn3a (see Xiang et al. 1996), whereas caudal rubrospinal neurons selectively express the C1q/TNF family member C1qL2. Within the caudal Brn3a+ C1qL2+ RN population, expression of the transcription factor Tshz3 is restricted to dorsomedial (cervically-projecting) RN neurons. Furthermore, the chemokine CXCL13 is expressed in a subset of dorsomedial RN neurons. In Tshz3 knockout mice, C1qL2 expression is decreased in dorsomedial RN neurons, but maintained in ventrolateral neurons, indicating distinct regulatory pathways for each RN subpopulation. Our analysis assigns topographic and molecular subdivisions to the rubrospinal projection pathway, and suggests that these distinctions contribute to the emergence of developmental order and to the mature function of this descending motor control system. We are currently mapping the connectivity of distinct RN subsets onto specific motor pools and evaluating the role of RN subset specific genes to the assembly of this descending motor control module.

PROTEIN TURNOVER OF THE WND/DLK KINASE IN AXONS REGULATES A RETROGRADE INJURY RESPONSE PATHWAY

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Axonal and synaptic morphology requires a conserved E3 ubiquitin ligase Hiw/Rpm-1/Phr1, which targets destruction of the Wallenda (Wnd)/DLK kinase. This MAPKKK regulates a nuclear signaling cascade whose mis-regulation in *hiw* mutants leads to dramatic synaptic overgrowth in *Drosophila*(1). Here we demonstrate that this signaling pathway regulates a transcriptional response to axonal injury, suggesting a relationship between injury/regeneration pathways and mechanisms that control synaptic structure. We developed an axonal injury and regeneration assay in *Drosophila* larval motoneurons, and describe a molecular reporter, the JNK phosphatase *puckered*, whose expression is induced by injury in a cell autonomous manner. Both injury signaling and the formation of new axonal branches at the injury site require Wnd, the JNK MAP Kinase, and the transcription factor Fos, the same pathway that promotes synaptic over-growth when *hiw* is mutant. Because axonal injury induces a nuclear response, and because axons are long, an important question is how the nucleus receives information from a distant injury site. The time required for *puckered* induction correlates with the distance of the injury site from the cell body suggesting that transport or localization of a component in axons may be a rate-limiting step to signaling. We find that the Wnd kinase localizes to axons and associates with vesicles that are rapidly translocated both anterogradely and retrogradely . and that functional axonal transport machinery is required for injury signaling. Injury leads to a rapid increase in the levels of Wnd protein in axons, concomitant with a decrease in Hiw protein. Increased levels of Wnd protein is sufficient to activate the injury signaling pathway. In *hiw* mutants, injury signaling is ectopically active, and neurons initiate a faster regenerative response to injury. Our results suggest that Wnd protein is continuously made, transported, and destroyed in axons, and that the regulation of protein turnover of Wnd, which activates a retrograde nuclear signaling cascade, is an important mechanism for detecting and responding to axonal damage.

The synaptic overgrowth phenotype of *hiw* mutants suggests that this injury response pathway can also regulate synaptic structure. Intriguingly, activation of this pathway in *hiw* mutants can counteract synaptic degeneration(2). Hence this regenerative injury response pathway may also be utilized to respond to synaptic stresses or signals.

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MOLECULAR MECHANISMS OF NETRIN-REGULATED SYNAPSE ASSEMBLY

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We recently showed that the canonical axon guidance cue Netrin instructs synaptic specificity (Colón-Ramos et al, 2007). This novel role of Netrin in synapse formation is conserved in vertebrates (Manitt et al., 2009). The molecular mechanisms by which the Netrin pathway mediates synapse formation are not understood.

We now show that the Netrin receptor, UNC-40/DCC, regulates synaptic assembly through an interaction with CED-10/Rac1, which then recruits MIG-10/lamellipodin to the presynaptic zones. The subcellular localization of MIG-10B organizes F-actin bundles at the Netrin-defined synaptogenic sites. This localized cytoskeletal rearrangement then results in the assembly of active zone complexes and synaptogenesis.

MIG-10 can be alternatively spliced into two isoforms: A and B. These alternatively spliced isoforms differ only by 21 amino acids at the N-terminus. We found that this small domain confers very distinct functional properties. MIG-10A is required downstream of Netrin for guidance and cell migration (Adler et al, 2006; Chang et al, 2006; Quinn et al, 2006). We now show that MIG-10B, but not MIG-10A, is both necessary and sufficient for presynaptic assembly downstream of Netrin. The unique N-terminal domain of MIG-10B is necessary for this protein to localize to presynaptic regions in an UNC-40/DCC dependent manner.

These results indicate that the neurodevelopmental outcome to the Netrin guidance cue is mediated by distinct MIG-10 isoforms. Our findings also provide mechanistic insights on how canonical guidance cues, such as Netrin, can organize the actin cytoskeleton to instruct presynaptic assembly and circuit formation.

VEGF GUIDES GRANULE CELL MIGRATION IN THE CEREBELLUM VIA VEGF RECEPTOR FLK1.

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VEGF regulates angiogenesis, but also has important, yet poorly characterized roles in neuronal wiring. Using several genetic and in vitro approaches, we discovered a novel role for VEGF in the control of cerebellar granule cell (GC) migration from the external granule cell layer (EGL) towards the Purkinje cell layer (PCL). GCs express the VEGF receptor Flk1, and are chemoattracted by VEGF, whose levels are higher in the PCL than EGL. Lowering VEGF levels in mice in vivo or ectopic VEGF expression in the EGL ex vivo perturbs GC migration. Using GC-specific Flk1 knock-out mice, we provide for the first time in vivo evidence for a direct chemoattractive effect of VEGF on neurons via Flk1 signaling. Finally, using knock-in mice expressing single VEGF isoforms, we show that pericellular deposition of matrix-bound VEGF isoforms around PC dendrites stimulates GC migration. These findings identify a previously unknown mechanism for VEGF in neuronal guidance.

EPHRINB2 STIMULATES GROWTH CONE REPULSION THROUGH A NOVEL PAK/NCK-DEPENDENT SIGNALING COMPLEX

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Eph receptors and ephrins play important roles in axon guidance during neuronal development by acting as contact-mediated guidance cues. However, the Eph Receptor-mediated forward signaling events that control axonal repulsion are still poorly understood. Using a reductionist approach in cultured primary cortical neurons, we identified Pak and Nck as essential components of a Rac/Cdc42-independent signaling complex that mediate ephrinB2-induced growth cone collapse (GCC). We find that this involves Pak1 kinase activity and an interaction between Pak and Nck, but not an interaction between Pak1 and Rac/Cdc42-GTP. Expression of either a Nck mutant that disrupts binding to Pak or a Pak mutant that disrupts binding to Nck dramatically reduces ephrinB2-induced GCC, suggesting that these proteins form a signaling complex with EphB receptors to regulate growth cone repulsion. Using cortical cultures from various EphB knockout and knock-in mice, we observed that EphB2 is the principle receptor required for ephrinB2-induced GCC in these cultured neurons. Consistent with the idea of a recruited signaling complex, we find that Pak1 co-immunoprecipitates with EphB2 in an EphB2 kinase-dependent manner. However, this interaction does not require Pak to bind Nck (a known EphB2 binding protein), suggesting a novel mechanism of interaction between EphB2 and Pak1. Not surprisingly, we find that RhoA-GTP and Rho kinase are required for ephrinB2-induced GCC in these neurons, but Rac- and Cdc42-GTP are not. Together, our data suggest that ephrinB2 binding to EphB2 induces the recruitment of a Pak/Nck signaling complex that transmits signals from the activated EphB2 receptors to cytoskeletal remodeling required for growth cone repulsion, suggesting a potential role for group I Paks in EphB-mediated axon guidance.

STABLE NEURON SUBTYPE-TAGGING AND GENE MANIPULATION IN THE CHICK SPINAL SENSORY-MOTOR CIRCUITRY

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The classical vertebrate embryology model, the chick (*gallus gallus*), is a potentially excellent subject for investigating spinal circuit assembly, because neuromuscular development and maturation are almost entirely completed during *in ovo* gestation. While early aspects of chick spinal cord development are readily accessible to molecular manipulation through *in ovo* electroporation, precise genetic investigation of later processes relevant for the functional maturation of spinal circuitries have so far been precluded. To alleviate these limitations, we designed a *Tol2*-transposon-based vector system achieving stable neuron subtype-specific transgene expression in the chick spinal cord. The system consists of a core element (*STEVE: stable-expression-vector*), based on the previously characterized zebrafish *Tol2* transposon, and two auxiliary vectors facilitating genomic integration and Cre/loxP-controlled gene activation. Herein, dual coupling of Cre recombinase and transposase expression to neuron subtype-restricted cis-regulatory elements achieves stable high-level bicistronic expression of epitope-tagged proteins and reporter fluorescent proteins facilitating axonal, synaptic, and electrophysiological tracing of genetically identified neurons. The system was successfully employed for the *in silico*-to-*in vivo* identification of novel cis-regulatory elements restricted to neuron subtypes in the late-gestation chick spinal cord and sensory ganglia; and facilitated in-depth study of central afferent projections at single-axon resolution. One of the identified 'late' elements drives expression in a novel subset of putative low-threshold mechanoreceptive sensory neurons with unique axonal termination zones in the E12 dorsal horn, and uncovered a previously unanticipated role of netrin family proteins in regulating central afferent connectivity.

EPHBS AND EPHRINBS CONTROL MIGRATION OF PROGENITOR CELLS IN THE ROSTRAL MIGRATORY STREAM

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A major group of neural progenitor cells originate in the subventricular zone and then migrate through the rostral migratory steam (RMS) to the olfactory bulb (OB). A number of molecules implicated in migration of neurons during development have been found in the RMS. These include sonic hedgehog, the Slit/Robo signaling pathway, PSA-NCAM and Ephs/ephrins. A number of lines of evidence in a diverse group of cell types indicate that EphBs and ephrin-Bs are potent factors in the control of cell migration, but their role in the control of migrating neural progenitors in the rostral migratory steam is not known. We used a combined approach of immunostaining, viral shRNAI knockdown, and viral CRE mediated knockout to examine the roles of EphB2 and ephrinBs in the regulation of cell migration. To knockdown expression of EphB2, we developed an in vivo shRNA knockdown strategy that enables us to test the requirement of specific EphBs in cell migration. Cells along the subventricular zone of adult mice were transduced with either EGFP and control shRNA or EGFP and shRNA directed against EphB2. Our data show that knockdown of EphB2 in neural progenitors disrupts their ability to properly migrate. Immunostaining reveals that migrating progenitors express the EphB ligand eprhin-B1, while neurogenic astrocytes ensheathing the progenitors express ephrin-B2. Further studies using CRE mediated knockout of ephrin-B1 and shRNA knockdown of ephrin-B2 validate the importance of these proteins in the migration of RMS progenitors. Together, these data support a role for EphB/ephrin-B interactions in the directed migration of progenitors and suggest that cell-to-cell interactions between progenitors and ensheathing astrocytes are critical for proper migration.

HETEROTRIMERIC G-PROTEIN SIGNALING IN ZEBRAFISH RETINAL AXON GUIDANCE.

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The growth cone navigates through a chemically complex environment by interpreting a balance of attractive and repellent guidance cues. However, to reach synaptic or intermediate targets, axons often must navigate through or near areas expressing axonal repellents. Our lab has shown that axons can modulate their response to multiple repellent cues through a G protein coupled, cAMP- and PKA- dependent signaling pathway, which we have termed the "antirepellent" pathway. Upon activation of G protein coupled receptors, associated G protein heterotrimers dissocate into two parts: the α subunit and the βγ complex. *In vitro*, inhibition of Gαi, Gαq or the βγ complex abrogates antirepellent signaling. In order to further investigate the role of heterotrimeric G protein signaling in axon guidance *in vivo*, we have taken advantage of the GAL4/UAS system to drive expression of dominant negative (DN) reagents in specific neuronal populations in the embryonic zebrafish. We have generated DN constructs which target the G protein alpha subunits Gαi, Gαq/11 as well as one which abrogates βγ signaling, GRK-CT. We also made a DN construct targeting GαS/olf, due to its canonical role as an activator of cAMP. We have generated stable transgenic lines for each UAS DN construct, as well as an Ath5:GAL4 line to drive expression in RGCs. Zebrafish RGC axons follow a well defined path, exiting the eye at the optic nerve head and crossing at the ventral midline to form the optic chiasm, before projecting to their synaptic targets in the contralateral tectum. RGCs expressing UASdnGαS make a major error and project to the ipsilateral tectum. Retinal axons expressing the DN constructs targeting Gαq/11 Gαi and βγ exhibit similar pathfinding defects near the midline. These results are consistent with the idea that GPCR signaling is important for axon pathfinding during normal development. The differing pathfinding defects displayed by DN GαS expressing axons versus those which express DNGαi/Gαq or DNβγ suggest that these related signaling components might work through distinct pathways to affect RGC guidance decisions near the chiasm. By examining the interaction between the DN-G-protein expressing axons and candidate GPCRs; we are working to identify novel receptors that influence axonal growth and guidance in the developing embryo. Thus, we are currently attempting to identify GPCRs that affect retinal midline crossing by searching for receptors whose knockdown potentiates DN-G-protein induced errors.

NRCAM DELETION CAUSES TOPOGRAPHIC MISTARGETING OF THALAMOCORTICAL AXONS TO THE VISUAL CORTEX AND IMPAIRS VISUAL ACUITY

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NrCAM, an L1 family axon guidance molecule (human chromosome 7q31.1 – 31.2) has been implicated in genetic association studies as a candidate susceptibility gene in autism spectrum disorders. Thalamocortical pathways may be compromised in autism contributing to abnormal visual processing. To investigate a role for NrCAM in thalamocortical axon targeting and visual responses, NrCAM null mutant mice were analyzed for topographic mistargeting of axons from the dorsal thalamus (DT) to distinct neocortical areas by retrograde tracing with lipophilic dyes. During development of the thalamocortical projection (embryonic day E14.5 to E16.5), NrCAM protein was localized to fibers projecting from the DT to the neocortex across the ventral telencephalon, an intermediate target of thalamocortical axons. During these stages NrCAM transcripts were enriched in the DT and cortical plate, declining with maturation. At E15.5, axons of thalamic neurons within the rostral DT of NrCAM null mutant embryos misprojected caudally within the ventral telencephalon, an intermediate thalamocortical axon sorting target, compared to wild type littermates. At postnatal day 7, when the thalamocortical map was complete, contingents of DT axons from rostrally located motor (ventro-anterior, ventro-lateral) and somatosensory (ventro-basal) thalamic nuclei of NrCAM null mice inappropriately targeted the visual cortex. NCAM null mutant mice showed normal patterning of neocortical areas and thalamic nuclei as evaluated by Nissl and serotonin staining. Thalamocortical topography in other members of the L1 family (L1, Neurofascin, and CHL1) was less affected. L1 and Neurofascin null mutant mice displayed a normal thalamocortical map, while CHL1 null mice exhibited misprojection of only somatosensory thalamic axons to the visual cortex (Wright et al.,2007).
To investigate whether NrCAM loss affects how the visual cortex responds to visual stimuli, we recorded visual evoked potentials (VEPs) in the binocular zone of the primary visual cortex in wild type and NrCAM null littermates at P27. NrCAM mutants displayed significantly reduced responses to visually-evoked potentials (VEPs) recorded from layer IV in the binocular zone of the primary visual cortex, particularly when evoked from the ipsilateral eye. These impaired visual responses indicated that loss of NrCAM decreases visual acuity and results in abnormal ocularity.
Our findings demonstrate that NrCAM is required for normal maturation of cortical visual acuity, and suggest that the aberrant projection of thalamic motor and somatosensory axons to the visual cortex in NrCAM null mutant mice impacts cortical function.

APP INTRACELLULAR DOMAIN ENHANCES NEURITE OUTGROWTH THROUGH ADENYLATE CYCLASE SIGNALING.

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Alzheimer's disease, the most common form of dementia in the elderly, is characterized by the progressive loss of synapses and neurons in the cerebral cortex and certain subcortical regions. Accumulation of β-amyloid peptides, generated via sequential proteolysis of amyloid precursor protein (APP), is a pathological hallmark of this neurodegenerative disorder. Despite of numerous studies conducted on APP and related β-amyloid pathology, the physiological function of APP still remains poorly understood. To investigate whether APP mediates intracellular signaling similar to many cell surface receptors, we expressed full-length APP or membrane-tethered APP intracellular domain (mICD) in primary mouse cortical neurons, immortalized rat hippocampal neurons (H19.7) and mouse N2a neuroblastoma cells. We observed that accumulation of APP C-terminal fragments following inhibition of APP processing by γ-secretase produces a significant enhancement of neurite outgrowth in N2a and H19.7 cells (343+/-25% and 282+/-28%, respectively, as compared to untreated cells; $p<0.001$). Similar increases were observed when APP mICD was expressed in N2a and H19.7 cells, and in cortical neurons (238+/-28%, 280+/-26% and 189+/-12% respectively, as compared to empty vector control). It is known that neurite outgrowth is intimately linked to cAMP-dependent and PI3K-dependent downstream signaling events such as PKA, CREB and GSK3β pathways. Base on this observation, we decided to examine more in details the signaling events that might be associated with APP-mediated neurite formation using cell signaling inhibitors (PKA: KT5720; PI3K: wortmannin; and adenylate cyclase: MDL 12,300A), and antibodies selective for phospho-PKA substrate Ser/Thr epitopes, phospho-CREB Ser133 epitope and phospho-GSK3β Ser9 epitope. We established that expression of mICD and accumulation of APP CTFs enhance activation of these pathways, which occurs upstream of the adenylate cyclase activation. Furthermore, we confirmed that APP mICD-mediated neurite outgrowth requires adenylate cyclase activation. Taken together, our results indicate that intracellular domain of APP is coupled to adenylate cyclase-dependent receptor-like signaling in neurons. We conclude that accumulation of APP or APP C-terminal fragments at the membrane could impact several brain functions such as neurite outgrowth, synaptic plasticity and memory formation, as a consequence of adenylate cyclase activation and subsequent activation of cAMP-dependent signaling cascades.
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ROLE OF THE SYNDIG FAMILY OF TRANSMEMBRANE PROTEINS IN AMPA RECEPTOR SYNAPTIC TARGETING

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Synaptic strength and plasticity is controlled in part by insertion and removal of AMPA receptors (AMPA-Rs) in the post-synaptic membrane. Knock-down of SynDIG1 in dissociated rat hippocampal neurons reduces AMPA-R content at developing synapses by ~50% as determined by immunocytochemistry and electrophysiology (Kalashnikova et al., Neuron, 2010, 65: 80-93). The magnitude of this effect matches that of the transmembrane AMPA-R associated regulatory proteins (TARPs) and PSD-95 identifying SynDIG1 as a previously unknown central regulator of postsynaptic AMPA-R targeting.

SynDIG1 co-immunoprecipitates with AMPA-Rs in heterologous cells and brain extracts. To dissect the mechanism of SynDIG1 function, potential AMPA-R-interacting sites in SynDIG1 were mutated and the effects on AMPA-R interaction and synaptic targeting measured in heterologous cells and in dissociated rat hippocampal neurons, respectively. SynDIG1-mediated synapse development is dependent on SynDIG1-association with AMPA-Rs via its extracellular C-terminus.

SynDIG defines a family of four proteins with distinct and overlapping expression in subsets of neurons. Preliminary studies indicate that SynDIG family members form homomeric and heteromeric complexes, suggesting that multiple SynDIGs might form complexes with AMPA-Rs. In addition, ongoing experiments document the effects of other SynDIG proteins in excitatory synapse development upon overexpression and knockdown in dissociated rat hippocampal neurons.

To investigate the function of SynDIG1 in vivo, a SynDIG1 conditional knockout mouse line was generated in which exon 4, which includes the transmembrane domain, is flanked by two LoxP sites (“floxed” allele, fl). Crossing fl/fl animals with Nestin-Cre transgenic mice results in viable mice with efficient knockout of the full length SynDIG1 protein in the brain. Preliminary studies suggest that AMPA-R distribution is changed in SynDIG1 deficient animals compared with controls. In addition, we are initiating electrophysiological analyses to document changes in AMPA-R function in SynDIG1 knockout animals compared with wild type controls.

Taken together, these studies provide important insight into the mechanism by which SynDIG family members influence AMPA-R targeting to synapses that regulate synaptic strength and plasticity.

GENERATION AND ANALYSIS OF NOGO RECEPTOR AND PIRB COMPOUND MUTANT MICE

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The regenerative capacity of injured adult mammalian CNS neurons is very limited. A major impediment to regenerative axonal growth and sprouting is the presence of inhibitory molecules associated with CNS myelin. Well-known inhibitors of growth include myelin-associated glycoprotein (MAG), the reticulon family member Nogo, and oligodendrocyte myelin glycoprotein (OMgp). Several neuronal cell surface receptors for myelin inhibitors of growth have been identified. Paired-immunoglobulin receptor B (PirB) and members of the Nogo receptor (NgR) family support binding of MAG, Nogo-66, and OMgp and have been shown to mediate inhibitory responses in primary neurons. Mechanistic studies of myelin inhibition have also revealed a high degree of redundancy in receptor systems that participate in signaling growth inhibition. Moreover, depending on the neuronal cell type examined, different receptors may be employed to signal inhibition. Here we report on the generation of Nogo receptor NgR1, NgR2, and NgR3 triple mutant mice as well as NgR1 and PirB double mutant mice. Mice deficient for all three NgRs or NgR1 and PirB are viable into adulthood and appear normal at the gross anatomical level. We are currently assessing neurite outgrowth inhibition of primary neurons isolated from wild-type and compound mutants. Cortical, cerebellar granule, dorsal root ganglion, and retinal ganglion neurons obtained from compound mutants are being tested for growth inhibitory responses toward crude CNS myelin or individual myelin inhibitors presented in substrate bound form. To study the role of myelin inhibitors in limiting axonal regeneration in the mature CNS, mutant mice will be subjected to optic nerve injury.

EMX1 REGULATES THE GUIDANCE OF THE CINGULATE PIONEERING AXONS OF THE CORPUS CALLOSUM

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Emx1 is a transcription factor expressed within the dorsal telencephalon. In this region, Emx1 is expressed in cortical projection neurons, a number of which project axons that comprise the largest fibre tract in the mammalian forebrain, the corpus callosum. To investigate a discrepancy in the literature regarding different *Emx1* mutant mice displaying different phenotypes, we have analysed callosal development in *Emx1* knockout mice backcrossed onto a C57Bl/6 background (to eliminate genetic predisposition to callosal phenotypes that are observed in some backgrounds). Prior to backcross, these mice were on a 129Sv strain and displayed complete absence of the corpus callosum in 100% of homozygous mutants (Qui et al., 1996). Immunohistochemical analyses of *Emx1* mice on the C57 background revealed small rostral Probst bundles in 100% of both embryonic and adult *Emx1* knockout mice, however more caudal regions of the corpus callosum were unaffected. Thus Emx1 appears to regulate the development of a subset of callosal axons. Further analyses of the Probst bundles in the *Emx1* knockout mice using high angular resolution diffusion imaging and tractography demonstrated that these misguided axons originate from the cingulate cortex. The cingulate cortex is the medial-most part of the dorsal telencephalon and contributes the pioneering axons of the corpus callosum. Pioneering axons express the guidance receptor Neuropilin1 and analysis of these axons using Neuropilin1 as a marker suggested a significant reduction of this receptor in the *Emx1* knockout mouse. These data suggest that Emx1 may play a specific role in regulating cingulate cortex axon guidance via regulation of Neuropilin1 expression. Specifically, the loss of Emx1 alters the presence of Neuropilin1 on cingulate pioneering axons which may then modify the response of these axons to the Neuropilin1 ligands, the semaphorins, which are expressed at the cortical midline.

THE ROLE OF EPHRINB3 IN AXON REGENERATION AND RECOVERY FROM SPINAL CORD INJURY

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Key experiments nearly 30 years ago demonstrated that CNS axon regeneration failure is due to an unfavorable environment in the CNS, rather than an intrinsic inability of CNS neurons to re-grow (1, 2). The inhibitory CNS environment is now documented to include chondroitin sulfate proteoglycans and myelin degradation products such as nogo, myelin associated glycoprotein and oligodendrocyte myelin glycoprotein.

EphrinB3 is a ligand of the Eph receptor family of receptor tyrosine kinases, which are implicated in the development of a variety of neural structures, including the corticospinal tract (CST) (3-6). EphrinB3 has been described as a myelin-derived axon growth inhibitor with a function parallel to Nogo, MAG and OMgp (7). Expression of ephrinB3 in myelinating olidodendrocytes and its receptor EphA4 on CST neurons could prove pivotal in determining the outcome of injury in the adult CNS. However, the role of ephrinB3 role in vivo had not been tested.

Following optic nerve crush, ephrinB3-/- mice had greater numbers of axons regenerating through the crush site, demonstrating a clear role in regenerative axon growth in vivo. We therefore decided to investigate the recovery of ephrinB3-/- mice following spinal cord injury. The spinal cord of ephrinB3-/- mice has developmental abnormalities, with multiple-midline-crossing CST fibers and a hopping gait due to interneuron misprojections (3-6). We have created near total spinal cord transections and dorsal hemisections in this strain. Post-injury performance after severe lesions is complicated during the initial month by increased spasticity in the ephrinB3-/- mice, but late recovery is significantly greater in mice lacking ephrinB3. EphrinB3-/- mice recovered to a significantly greater extent than wild type littermates in behavioral tests following bilateral thoracic hemisection. CST regeneration was detected in mutant animals, with significantly increased numbers of axons traversing the lesion site and growing caudally into the spinal cord grey matter. Thus, ephrinB3 expressed largely by olgodendrocytes plays a role in limiting axonal regeneration in the adult CNS.

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MEGF8 IS A NOVEL GENE REQUIRED FOR DEVELOPMENT OF THE SOMATOSENSORY SYSTEM, EYE, HEART, AND LIMB

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A third-generation forward genetic screen in mice using the chemical mutagen ENU was performed to discover novel receptors and ligands that orchestrate development of the mammalian peripheral nervous system. One of the lines that emerged from this recessive screen, Line 687, exhibits defasciculation of the ophthalmic branch of the trigeminal nerve, which provides sensory innervation to the face. In addition, axonal projections from the dorsal root ganglia (DRG) are undergrown and poorly branched. In addition, homozygous mutants exhibit profound developmental abnormalities of the heart, severe polydactyly, and die at approximately E16.5. The mutation in Line 687 was mapped to a single missense mutation in the novel gene *Multiple-EGF-like-domains-8 (Megf8)*. *Megf8* encodes a putative transmembrane protein, with a large extracellular domain that contains EGF repeats, Plexin-Semaphorin-Integrin-like domains, a CUB domain, and Kelch domains. The mutation found in Line 687 results in a Leucine to Proline substitution in one of the Kelch domains of *Megf8*. In situ hybridization experiments show that *Megf8* is expressed throughout the nervous system during development, and particularly high levels of expression are present in the DRG, trigeminal ganglia, sympathetic chain ganglia, and developing neuroepithelium. In order to further study *Megf8's* function we generated a *Megf8* conditional knock-out mouse line. When the *Megf8* conditional line is crossed to a germline Cre line, so as to ablate *Megf8* in all cells, the ophthalmic branch of the trigeminal ganglion is defasciculated and spinal nerves are underdeveloped, as was observed in Line 687 mutant embryos. However, when the conditional line is crossed to Wnt1-Cre to ablate *Megf8* in all cells derived from the neural crest lineage, the trigeminal defect becomes more pronounced and is accompanied by degeneration of the eyes. Spinal nerves are also underdeveloped in the *Megf8/Wnt1-Cre* conditional line, which suggests a cell autonomous function of *Megf8* in the developing DRG. Thus, *Megf8* is a novel gene required for development of the axonal projections of somatosensory neurons as well the eye, heart, and limb.

VEGF SIGNALLING THROUGH NEUROPILIN 1 GUIDES COMMISSURAL AXON CROSSING AT THE OPTIC CHIASM.

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During development, the axons of retinal ganglion cell (RGC) neurons must decide whether to cross or avoid the midline at the optic chiasm to project to targets on both sides of the brain. Whilst repulsive signals are known to be important for the formation of the ipsilateral projection, as yet no midline factor that helps RGC axons project contralaterally has been identified. By combining genetic analyses with in vitro assays we have found that neuropilin1 (NRP1) is essential for contralateral growth of RGC axons at the mouse optic chiasm.
We found that NRP1, but not NRP2, is expressed by mouse RGC axons as they extend through the optic chiasm and that NRP1 is required for normal optic tract organisation and RGC axon crossing at the chiasm midline. Unexpectedly, we discovered that this essential role for NRP1 in chiasm development was due to its ability to serve as a receptor for VEGF164, a neuropilin binding isoform of VEGF-A best known for its ability to stimulate endothelial cell proliferation and migration during blood vessel growth. Thus, mice lacking VEGF164, but not class 3 semaphorin signalling, the traditional neuropilin ligands in the nervous system, develop ectopic ipsilateral projections. This requirement for VEGF164 in contralateral guidance at the optic chiasm is independent of VEGF-A's well characterised role in patterning blood vessels: mice lacking NRP1 specifically in blood vessel endothelium display blood vessel defects comparable to full NRP1 knockouts but contain a normal proportion of ipsilaterally projecting RGCs. Instead, we found that VEGF164 acts on the NRP1-expressing RGC axons as a growth promoting and chemoattractive guidance signal. Beyond their significance for understanding axon wiring in the visual system, these findings provide the first evidence that VEGF-A is a physiological axon guidance cue with a key role in commissural axon guidance.

ROUNDABOUT RECEPTORS AND THE EVOLUTION OF AXON GUIDANCE RECEPTOR FUNCTIONAL DIVERSITY

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The cognitive, behavioral, and sensory complexity of modern animals depends on sophisticated control of axon guidance decisions throughout development. An increase in nervous system complexity over evolutionary time has been facilitated by the functional diversification of axon guidance molecules. A prime example is the Roundabout (Robo) family of axon guidance receptors: while the canonical role of Robo receptors is to mediate axon repulsion in response to the secreted midline repellant Slit, additional family members exhibiting diverse guidance activities are present in multiple animal groups. While in some cases these additional activities are similar (for example, both Robo3/Rig-1 in vertebrates and Robo2 in flies can promote midline crossing in some contexts), it is unclear whether they represent common ancestral functions or if they have arisen independently in multiple lineages.

We have previously shown that the functional diversity of *Drosophila* Robo receptors is made possible by the modular nature of receptor ectodomains, and is specified by structural differences between individual receptor immunoglobulin-like (Ig) domains. Now, we examine the evolutionary origin of this functional diversity through phylogenetic and functional analyses of Robo receptor family members in other species. Phylogenetic analyses reveal that the Robo receptor family has expanded from a single ancestral gene independently in insects and vertebrates, and support the independent acquisition of diverse guidance activities in these groups.

In addition, the recent expansion of the Robo receptor family in the *Drosophila* lineage makes functional comparisons possible with insects that have retained an ancestral complement of receptors. We present functional analyses of Robo receptors from one such insect, the flour beetle *Tribolium castaneum*. Robo receptors from *Tribolium* can respond to *Drosophila* Slit and mediate midline repulsion in flies, but are unable to recapitulate the lateral positioning activities of *Drosophila* Robo2 and Robo3, perhaps indicating that the latter activity arose subsequent to the divergence of the beetle and fly lineages. Our studies therefore provide insight into the molecular and evolutionary origins of Robo receptor functional diversity.

RECYCLING ENDOSOMAL RABS REGULATE RETINAL AXON ELONGATION IN VIVO

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In Xenopus, the retinal axon projection to the tectum has been a useful model to unravel some of the molecular mechanisms controlling axon elongation and pathfinding. Endocytosis is involved in the growth cone's response to various guidance cues and is crucial for the continuous reshaping of the growth cone necessary for its steering and advance. To test whether the intracellular trafficking pathway within the growth cone may determine the specific sub-cellular functions served by endocytosis, we are studying the role of several Rabs expressed in the developing retina. Rabs are small GTPases, which selectively associate with different vesicle compartments, including endosomes, to regulate their biogenesis, transport and fusion. Briefly, many molecules after internalisation transit through the Rab5 positive early/sorting endosomes before either being sent back to the membrane via Rab4 and/or Rab11 recycling endosomes or targeted to Rab7 late endosomes for degradation.
We can detect and monitor the dynamics of Rabs5 endosomes in Xenopus growth cones using antibodies and GFP/RFP fusion proteins. Retinal axons over-expressing constitutively active (CA) Rab5 mutants in vivo navigate correctly to the tectum, however, their arrival at the tectum is significantly delayed. We then asked which of the recycling or degradative pathways downstream of Rab5 leads to this phenotype. As expected, Rabs4, 11 and 7 positive endosomes are found in growth cones. Expression of dominant negative (DN) or CA forms of these three Rabs, like CA Rab5, did not noticeably affect long-range pathfinding, but DN Rab4 and 11 caused delayed arrival of axons at the tectum. Both CA Rab5 and DN Rab 4 lead to a decreased axon extension rate in vitro (by 33% and 25% respectively). Consistently, DN Rab4 expression impairs growth cone advance along the optic tract in vivo. Finally, live monitoring of Rab4 and 5 endosomes and acute blockage of recycling in vitro support a direct contribution of growth cone local recycling to axon elongation. All together, this suggests that local recycling via Rab5 and 4 within the growth cone control the rate of axon extension.

PATTERNS OF AXON FASCICULATION IN THE DIAPHRAGM: A NOVEL MODEL OF LEFT-RIGHT ASYMMETRY

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Several cases of anatomical, functional and molecular Left-Right asymmetric features have been described in the vertebrate brains, while in contrast no evidence has yet been provided on the existence of a L-R asymmetric identity of spinal cord neurons and connectivity. The diaphragm is a respiratory muscle comprising a central tendinous region and two lateral muscles innervated by ipsilateral pools of cervical motoneurons forming the left (L) and right (R) phrenic nerves. Although symmetric in appearance at coarse level, both lateral muscles and phrenic nerves exhibit left-right (L-R) asymmetric features. Our quantitative analysis indicates first that L and R muscles differ in size, the R muscle being larger. Second, while the L phrenic nerve divides into two fascicles making a T-shape, the R nerve splits into several fascicles irradiating from the entry point in a fan shape. Overall, the L and R nerves significantly differ in the degree of defasciculation and length of secondary axon fascicles. These differences are present from the onset of target inervation, suggesting possible contribution of the symmetry-breaking Nodal signaling setting L/R asymmetry of the visceral organs in the early embryo. To assess this possibility we studied embryos lacking Rfx3, a transcription factor controlling ciliogenesis whose deletion impairs the normally L restricted Nodal signaling leading to defect in visceral L-R asymmetry. In Rfx3 null embryos, both muscles width and nerve defasciculation patterns become symmetric. Interestingly, while the L muscle enlarges to adopt a R morphology, the L axon pattern is unchanged. Conversely, the R muscle is not affected whereas the R nerve adopts a L-like fasciculation pattern. Such uncoupling between muscle and nerve components strongly suggests that L/R innervation features are encoded by phrenic motoneurons. In an effort to identify differentially expressed L/R nerve factors, we undertook a proteomic screen of protein contents in neonatal L and R phrenic nerves. In parallel, we investigated know regulators of fasciculation and report a contribution of metalloproteinase members in the establishment of the patterns of axon fasciculation.

A LIVE *DROSOPHILA* MODEL OF AXONAL INJURY AND DEGENERATION

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Axonal degeneration is a prominent feature of spinal cord injury and many neurological disorders including Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis. Studies of the Wallerian degeneration slow (Wlds) mouse indicate that axonal degeneration is an active process. It has been more than two decades since the Wlds mutation was discovered, however, the self-destruction mechanisms by which axons degenerate remain elusive.

In order to identify novel players that control axonal degeneration, it is desirable to perform unbiased, large-scale genetic screens. However, such screens are technically difficult, costly, and time-consuming in conventional rodent models. *Drosophila* has been proven an exceptionally powerful system to study human diseases. Since Wallerian degeneration is conserved from insects to vertebrates, we sought to model nerve injury in flies. In this study, we have established a reproducible, live *Drosophila* model of axonal injury and degeneration, which appears suitable for large-scale screens.

We initially examined flies that express Green Fluorescent Protein (GFP) to highlight selective nerves. We found that the sensory nerve along the anterior wing margin provides an excellent system for axotomy: 1) the fly wings are semi-transparent, so that the wing nerve can be visualized directly on live flies with GFP; 2) the wing nerve forms a stereotypical nerve bundle in the wing vein, allowing precise and reproducible axotomy; 3) each fly has two wings, so it is possible to compare the injured and intact wing nerves on the same fly overtime; and, 4) the wings are dispensable for survival, in which case, for genes that are critical for survival or normal development, we can manipulate and test them specifically in the wings.

Using this novel model of axonal injury and degeneration, we have performed a pilot screen. Whereas normally axonal degeneration is rapid, select lines display a mild to moderate delay in axonal degeneration, one of which appears to be an upregulation line of Nmnat (nicotinamide mononucleotide adenylyltransferase). Nmnat is the major component of the Wlds gene mutation and alone is sufficient to protect axons. Thus, this model of axonal degeneration has proven effective. Future plans include expanding the screen to a larger scale as well as verifying and studying initial candidate genes. By defining such genes and investigating their functions and mechanisms, we aim to draw a fuller picture of genes and signaling pathways involved in axonal degeneration, and how they interact to control this process.

ATLASTIN CONTROLS ZEBRAFISH MOTILITY AND SPINAL MOTOR AXON ARCHITECTURE VIA INHIBITION OF THE BMP PATHWAY

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The development of connections in the nervous system depends on the critical ability of growing axons to find their appropriate and often distant targets. Recent studies have shown that major classes of secreted morphogens including the Bone Morphogenetic Proteins (BMP), known to specify cell fates during early embryogenesis, are also involved in axon guidance and synaptic growth at later stages of neural development. Interestingly, concomitant analyses in invertebrate models have recently unveiled a link between the BMP pathway and neurodegenerative disorders affecting upper or lower motor axons. To achieve significant progress in the understanding of motor neuron degeneration, we performed lack- and gain-of-function analyses of a protein involved in hereditary spastic paraplegia (HSP) during development of the zebrafish.
HSP is a heterogeneous group of neurodegenerative disorders characterized by progressive spasticity of the lower limbs due to degeneration of the cortico-spinal tracts. We have shown that the knockdown of Atlastin, a protein involved in a major juvenile form of HSP, causes a drastic decrease in larval mobility associated with abnormal architecture of spinal motor axons and a significant up-regulation of the BMP signaling pathway. The specificity of Atlastin knockdown phenotype was confirmed by rescue analyses with human Atlastin and transplant experiments. Furthermore, overexpression analyses have revealed that Atlastin functions as an inhibitor of the BMP/Smad pathway. Primary cultures of zebrafish spinal neurons have shown that Atlastin partially co-localizes with type I BMP receptors to late endosomes, suggesting that Atlastin may regulate BMP receptor trafficking. Finally, we have demonstrated that a genetic or pharmacological inhibition of the BMP pathway rescues the loss of mobility and spinal motor axon defects of Atlastin knockdown, emphasizing the importance of fine-tuning the balance of BMP signaling for vertebrate motor axon development.

REGULATION OF CALCIUM HOMEOSTASIS IN GROWTH CONE MOTILITY

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Calcium signaling has long been known to be crucial to growth cone motility in axon guidance. A pivotal component of calcium regulation is store operated calcium entry, a poorly understood process within growth cones. We have evidence to suggest that the calcium regulatory proteins Homer and Stromal Interacting Molecule 1 (STIM1) both act to regulate store operated calcium entry within navigating growth cones. Homer proteins are post-synaptic density scaffold proteins with known functions in calcium homeostasis. STIM1 is an exquisite calcium-sensing protein, located in the plasma and endoplasmic reticulum membranes. The function of STIM1 in developing neurons is unknown. Using protein knockdown in neurons from embryonic dorsal root ganglia and a growth cone turning assay, we demonstrated that both Homer and STIM1 are required for growth cone navigation in axon guidance. Knock down of Homer1 or STIM1 converted growth cone turning from attraction to repulsion in response to the calcium dependent guidance cue brain derived neurotrophic factor (BDNF). For example, knockdown of STIM1 induced a switch in growth cone turning, from chemoattraction towards BDNF (turning angle 10.64 ± 1.62 degrees) to chemorepulsion (-7.02 ± 2.64 degrees). Our data implicate both Homer1 and STIM1 in calcium regulation within the growth cone. Supporting our hypothesis, knock down of Homer1 had no effect on growth cone repulsion in response to the calcium independent guidance cue, semaphorin-3a. Surprisingly, the normal chemorepulsive response to semaphorin-3a (-7.72 ± 2.37 degrees) was abolished by STIM1 knockdown (0.95 ± 2.48 degrees). This recent data suggests multiple roles for STIM1 and store operated calcium entry in developing neurons. Deciphering the underlying homeostatic mechanisms that control calcium in the growth cone, and allow calcium to signal multiple, discrete events has direct implications for a wide variety of developmental and neurodegenerative conditions.

THE ROLES OF EPH/EPHEXIN SIGNALING AND $CA_V2.1$ CALCIUM CHANNELS DURING SYNAPTIC HOMEOSTASIS

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At the Drosophila neuromuscular junction (NMJ), inhibition of postsynaptic glutamate receptor function initiates a homeostatic increase in presynaptic neurotransmitter release. This increase in release offsets the impaired receptor function and restores muscle excitation. We previously uncovered a presynaptic signaling system required for this homeostatic response at the NMJ. This system consists of the Eph receptor, Ephexin (Rho-type guanine nucleotide exchange factor), Rho-type GTPases, and $Ca_V2.1$ calcium channels. We postulate that cytoplasmic signaling components like Ephexin and Cdc42 (Rho-type GTPase) couple synaptic Eph signaling to the modulation of presynaptic $Ca_V2.1$ function to enhance neurotransmitter release. These data are being extended in several ways. Homeostatic signaling is bi-directional at the Drosophila NMJ. Preliminary data suggest that some components of this signaling system are also necessary for an accurate homeostatic decrease in presynaptic release. Additionally, data will be presented from an ongoing genetic approach to identify new signaling components, enhancing our understanding of synaptic homeostasis, Eph/Ephexin/RhoGTPase signaling, and calcium channel modulation.

ADAPTATION AND RESENSITIZATION OF RETINAL GROWTH CONES ON SUBSTRATE-BOUND EPHRIN PATTERNS

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Axons are guided to their appropriate targets by the interaction of extracellular guidance molecules with membrane-anchored sensors. In-vitro assays have shown that neuronal growth cones can adapt to such guidance signals. Using microcontact printing we produce various patterns of substrate-bound ephrin-A5 and investigate adaptation mechanisms of chick retinal axons.

Our results demonstrate that chick retinal growth cones can desensitize and resensitize to repulsive ephrin-A5 signals: (i) soluble ephrin-A5 initially causes the collapse of temporal growth cones. However, 80% of these growth cones recover and resume migration in the presence of soluble ephrin-A5 after 60 min. (ii) Temporal axons can grow out from a retinal explant in the presence of soluble ephrin-A5, but no longer respond to substrate-bound ephrin-A5 patterns. (iii) Initial axonal outgrowth is observed when retinal explants are placed on substrate-bound ephrinA5. Here growth cones are protected against collapse induced by soluble ephrinA5. (iiii) On 'gap patterns', where axons initially grow out on substrate-bound ephrinA5 and reach an ephrin-free gap of laminin before they are again confronted with ephrinA5, temporal axons resensitize depending on the size of the gap.

We are currently investigating the molecular mechanisms involved in desensitization and resensitization. In principle, these mechanisms could be regulated directly at the receptor level or further downstream in the signalling cascade. Experiments using dynasore suggest that endocytosis of Eph-receptors is not involved in desensitization to ephrin-A5. In addition, treatments with anisomycin and cycloheximide indicate that local protein synthesis does not seem to play a role neither in initial growth cone collapse in response to ephrin-A5 nor in desensitization or resensitization to ephrin-A5. In contrast, preliminary results show that dephosphorylation is involved in desensitization. After addition of the phosphatase inhibitor vanadate temporal axons lose their ability to grow out in the presence of soluble ephrin-A5 and don't recover after an ephrin-A5 induced collapse. In addition, temporal axons are unable to grow out on substrate-bound ephrin-A5 in the presence of vanadate. In summary, our findings indicate that de-phosphorylation of Eph-receptors, possibly by the transmembrane phosphatase PTPRO, might be involved in desensitization and resensitization of chick retinal axons to ephrin A.

TAG1 REGULATES THE MEMBRANE ORGANISATION AND ENDOCYTOSIS OF THE SEMAPHORIN3A RECEPTOR COMPLEX

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The neural cell adhesion molecule, transient axonal glycoprotein (TAG1 or CNTN2), is found on many developing axonal tracts, including the commissural axons of the spinal cord and sensory neurons from the dorsal root ganglia. However, despite its discovery two decades ago, the mechanism by which TAG1 acts to control cellular signalling events in axon guidance is still elusive. Our lab has previously found that TAG1 is required for DRG neurons to respond to Semaphorin3A (SEMA3A), and mutations in TAG1 lead to aberrant guidance of sensory afferents into the spinal cord (Law et al 2008. Development, 135, 2361-71). This work suggested that TAG1 in some way controls the endocytosis of the SEMA3A receptor complex, but the mechanism by which this is done was not clear. We now report that TAG1 plays a key role in organising the components of the SEMA3A receptor, Neuropilin1 (NRP1), PlexinA4 (PLXNA4) and L1 (L1CAM), in the neuronal plasma membrane. Specifically, TAG1 interacts directly with NRP1 and forms a constitutive complex with both NRP1 and L1. SEMA3A treatment, however, changes the nature of these interactions and the disposition of the receptor components in lipid rafts in a TAG1-dependent manner. The differential recruitment of the receptor components to different membrane subdomains results in their internalisation via independent endocytic pathways. Thus, TAG1 regulates the Semaphorin3A signalling cascade and has emerged as an important player in modulating the responses of neurons to SEMA3A through its ability to modulate the disposition of key receptor components on the cell membrane.

ACTION POTENTIALS DRIVE BODY WALL MUSCLE CONTRACTIONS IN CAENORHABDITIS ELEGANS

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The sinusoidal locomotion exhibited by C. elegans predicts a tight regulation of contractions and relaxations of its body wall muscles. Skeletal muscle contractions are known to be driven by voltage-gated sodium channel-dependent action potentials [1]. How coordinated motor outputs are generated and regulated in C. elegans, which does not encode voltage-gated sodium channels [2] remains mysterious. We present evidence that C. elegans body wall muscles do fire regenerative, calcium-dependent action potentials that are driven by the L-type voltage-gated calcium (EGL-19) and Kv1 family voltage-dependent potassium (SHK-1) channels. These action potentials are physiologically relevant, as a single action potential is essentially to induce a brief muscle contraction, whereas a train of action potentials drives a sustained period of muscle contractions. We further demonstrate that the excitatory and inhibitory motoneuron activities regulate the frequency of action potentials to coordinate muscle contraction and relaxation, respectively. This study not only confirms the dual modulatory model of the C. elegans locomotory circuit [3], moreover, further indicates that muscle cells can integrate graded inputs of the nervous system [4], but fire quantal electrical signals to facilitate regulated locomotion.

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REGULATION OF MICROTUBULE CYTOSKELETON DYNAMICS IN *C. ELEGANS* AXON REGENERATION

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A major question in axon regeneration studies is that of how axon injury transforms the stable MT cytoskeleton of the mature axon to the dynamic MT cytoskeleton of a regrowing axon. We are using *C. elegans* mechanosensory neurons to analyze the effects of laser axotomy on microtubule dynamics in single axons. To visualize changes in MT growth and maintenance we tagged the MT end-binding protein EBP-2 with GFP. We find that prior to injury PLM axonal processes have relatively stable microtubules, as compared to the cell body region. Laser axotomy of the axon causes an acute loss of EBP-2 comets, suggesting that MTs are destabilized in response to axonal injury. In the wild type, severed axons commence regrowth after a lag period of 3-6 h. During this period we observed a dramatic local upregulation of plus end dynamics near the site of injury. Following initiation of regenerative growth, MT dynamics are globally upregulated in the injured neuron. Loss of function in the MT end binding protein EBP-1 blocks axon regrowth, suggesting MT dynamics influence efficient regrowth.

The DLK-1 MAPK cascade is critical for growth cone formation after axonal injury (Hammarlund et al 2009; Yan et al 2009). We find that loss of DLK-1 specifically alters the acute changes in MT dynamics of the axonal response to injury. We propose that in response to axonal injury the DLK-1 cascade changes the state of the MT cytoskeleton to enhance MT dynamics. We are investigating the mechanism by which the DLK-1 kinase and other MT regulating kinases act on the MT cytoskeleton in regrowing axons.

INVESTIGATING THE ROLE OF SLIT/ROBO SIGNALING IN NEURONAL MORPHOGENESIS DURING POSTNATAL BRAIN DEVELOPMENT.

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Neuronal morphogenesis is a critical step in the formation of neural circuits and requires the elaboration of both axonal and dendritic branches. The Slit family of axon guidance molecules and their Robo receptors have been shown to regulate axonal branching during embryonic development in a variety of organisms and cell types, including zebrafish retinal ganglion cells, Drosophila space-filling neurons, and mouse sensory neurons. Additionally, in vitro studies have implicated Slit/Robo signaling in dendritic branching in both mouse cortical neurons and Xenopus retinal ganglion cells. Slits and Robos continue to be expressed in the mouse brain after birth, suggesting a role in postnatal development of the central nervous system (CNS). However, owing to the redundancy of the genes and the lethality of compound mutants, the precise role of Slit/Robo signaling in postnatal development has not been established. To overcome these genetic obstacles, we have generated a homozygous *Robo1*$^{-/-}$;*Robo2*$^{flox/flox}$ mouse to investigate the function of the Robo receptors in a variety of neuron populations during the development of the central nervous system. Excision of the *Robo2*^{flox} allele is achieved by targeted injection of adeno-associated viruses (AAVs) into the neonatal mouse brain to selectively express Cre recombinase in a region and cell-type specific fashion. By choosing the appropriate serotype of AAVs and the anatomical location of the injection, we are able to target a variety of CNS neurons, including neocortical pyramidal neurons, cerebellar Purkinje cells, and retinal ganglion cells. By controlling the titer of the viruses and coexpressing fluorescent reporters, neurons can be sparsely labeled, allowing 3D tracing and morphometric analysis. Using these tools and techniques, we are investigating the morphological changes of those neurons with the selective loss of Slit/Robo signaling. These studies will complement the conventional genetic approach using Cre-driver mice with neuron-specific promoters and allow us to establish the precise role of Slit/Robo signaling in the formation of stereotypic axonal and dendritic branches. Neonatal viral injections to deliver Cre recombinase and label single cells are also likely to be useful for studying other lethal genes in a region-specific fashion and may generate further insight into the relationship between neuronal branching and other postnatal developmental processes, such as dendritic spine formation and synaptogenesis.

FGF8 EXPRESSED BY THE MOUSE COMMISSURAL PLATE REGULATES FORMATION OF THE CORPUS CALLOSUM AND HIPPOCAMPAL COMMISSURE

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Three major axonal tracts (or commissures) form the principal connections between the left and right forebrain hemispheres: the corpus callosum, hippocampal commissure and the anterior commissure. In humans, simultaneous malformation of multiple forebrain commissures is associated with a wide spectrum of congenital disorders. This occurrence suggests common mechanisms may underpin the development of these three forebrain commissures.

Three-dimensional visualisation of the forebrain commissures using diffusion tensor magnetic resonance imaging (DTMRI) and immunohistochemistry identified a common anatomical region known as the commissural plate where commissural axons cross the midline early in development (Moldrich et al., J. Comp. Neurol., 2010). This precise anatomical arrangement may indicate that the commissural plate expresses molecules that guide forebrain commissural axons across the midline.

One candidate molecule expressed in the commissural plate is Fibroblast growth factor 8 (FGF8). Throughout interhemispheric midline development, *Fgf8* is predominately expressed by midline glial populations that border the developing corpus callosum and hippocampal commissure.

To test the hypothesis that FGF8 acts as a guidance cue for axons of the corpus callosum and hippocampal commissure, we utilised an *in vitro* collagen gel assay. In the mouse, the cingulate cortex projects pioneering axons of the corpus callosum to the midline at E15.5. These pioneering projections are then followed by projections from the neocortex at E17 to form the bulk of the corpus callosum, while the hippocampal commissure is pioneered by axonal projections from the hippocampus at E16. A point source of diffusing recombinant FGF8 was therefore paired with E15.5 cingulate cortex, E17 neocortex, and E16 hippocampus. Recombinant FGF8 induced significant attractive guidance of both pioneering axons of the cingulate cortex and hippocampus, but not neocortical neural projections. Co-ordinately, conditional knockdown *in vivo* of FGF8 signalling in radial progenitors in $Fgf8^{flox/flox};Nestin^{Cre}$ mice disrupts corpus callosum and hippocampal commissure formation. These findings suggest that FGF8 expressed by midline glia in the mouse commissural plate regulates both corpus callosum and hippocampal commissure formation.

SINGLE-CELL OPTOGENETIC EXCITATION DRIVES HOMEOSTATIC SYNAPTIC DEPRESSION

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Homeostatic processes have been proposed to explain the discrepancy between the dynamics of synaptic plasticity and the stability of brain function. Forms of synaptic plasticity such as long-term potentiation (LTP) alter synaptic activity in a synapse- and cell-specific fashion. Although network-wide excitation triggers compensatory homeostatic changes, it is unknown whether neurons initiate homeostatic synaptic changes in response to cell-autonomous increases in excitation. Here we employ optogenetic tools to cell-autonomously excite CA1 pyramidal neurons and find that a compensatory postsynaptic depression of both AMPAR and NMDAR function results. Elevated calcium influx through L-type calcium channels leads to activation of a pathway involving CaM kinase kinase and CaM kinase 4 that induces synaptic depression of AMPAR and NMDAR responses. The synaptic depression of AMPARs but not of NMDARs requires protein synthesis and the GluA2 AMPAR subunit, indicating that downstream of CaM kinase activation divergent pathways regulate homeostatic AMPAR and NMDAR depression.

GENETICALLY-DEFINED LINEAGE TRACING OF NKX2.2-EXPRESSING CELLS IN CHICK SPINAL CORD

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In the neural tube, generation of neurons and glial cells is dependent on the domain structure which is defined by the expression of specific transcription factors. In the neural tube, different classes of neurons generate from different 'domain structure' defined by the expression of transcription factors. Recently, it was reported that these transcription factors that define positional identity also regulate subtype-specific development of astrocytes. However, the mechanism that governs glial cell development largely remains unknown as compared with those of neuron.

To analyze developmental mechanisms in the specific cell lineage more easily, we established a novel method to permanently introduce exogenous gene into a specific cell type using chick embryos. Murine retrovirus is widely used for the lineage tracing experiment in mice. Chick cells cannot be infected by murine retrovirus because they lack murine retroviral receptor, CAT1 (Cationic aminoacid transporter1) at the plasma membrane of the cells. We introduced CAT1 gene by electroporation followed by injection of murine retrovirus. By using this method, we successfully transduced murine retrovirus into chick neural tube. We analyzed cell lineage from the p3 domain by restricting CAT1 expression by nkx2.2-enhancer and found that most of the labeled cells became oligodendrocytes when the cells were labeled at cE4. Moreover, the labeled oligodendrocytes were found all over the white matter in spinal cord including the most dorsal spinal cord. We will present on recent data in this poster.

THE *SPACE CADET* GENE REVEALS A CRITICAL ROLE FOR THE RB1 TUMOR SUPPRESSOR IN RETINAL AXON GUIDANCE

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Before they navigate towards the CNS midline, retinal ganglion cell (RGC) growth cones must first exit from the retina. While the netrin/DCC pathway has been shown to be critical for RGC growth cones to exit from the eye, the mechanisms that guide RGC growth cone towards the retinal exit point remain largely unknown. We had previously shown that mutants of the zebrafish *space cadet* gene display pathfinding at the CNS midline, but also fail to exit from the retina (Lorent et al, 2001, *Development*). To examine the role of space cadet during intraretinal guidance in more detail, we labeled small, discrete groups of RGCs to assess of axonal behaviors within the retina. We observed two intraretinal guidance defects in *space cadet* mutants. First, in ~64% of mutant retinas, RGC axons navigate correctly towards the exit point, but then bypass the exit point and continue extending ectopically within the retina. Second, in ~26% of mutant retinas, RGC axons immediately mis-orient and project to ectopic regions of the retina. Importantly, histology and marker analysis reveals that the overall retinal cyto-architecture is intact, and that retinal ganglion and exit glia cells are present in appropriate numbers and are properly specified, demonstrating that *space cadet* plays a critical role in intraretinal axonal guidance. Furthermore, chimera analysis shows that *space cadet* activity is required cell autonomously within RGCs. Surprisingly, positional cloning reveals a premature non-sense mutation in the retinoblastoma (Rb1) gene, truncating the Rb protein within the Cyclin domain. Rb1 is a well-characterized tumor suppressor gene, and conditional deletions in mouse gene have revealed reduced retinal cell numbers, mostly due to apoptosis, yet a role for Rb in retinal axonal guidance has not been reported. We will provide further data to determine how *space cadet*/ Rb affects retinal axon guidance.

THE MITOTIC REGULATORY PROTEIN, NPP-17/RAE1, INTERACTS WITH PHR PROTEINS TO REGULATE AXON TERMINATION

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Pam/Highwire/RPM-1 (PHR) proteins are conserved from *C. elegans* to mammals, and play a critical role in synapse formation, and axon outgrowth and termination. In *C. elegans*, RPM-1 functions as an E3 ubiquitin ligase to negatively regulate the DLK-1 MAP kinase pathway (1). RPM-1 also acts as a positive regulator of a Rab GTPase pathway (2). Here we report the identification of a novel RPM-1 binding protein, Nuclear Pore Protein (NPP)-17/RNA Export protein (Rae)-1. Previous studies in mammals have shown that Rae1 is a microtubule binding protein that acts as an essential cell cycle regulator. While Rae1 is expressed in neurons, its postmitotic function remains unknown. We now provide evidence of a critical postmitotic role for NPP-17/Rae1 in regulation of neurodevelopment, specifically axon termination in *C. elegans*.

Using a heterologous expression system, we have found that human Pam binds to rat Rae1. We have further mapped this interaction to a conserved protein domain in the PHR proteins that we call the Rae1 binding domain (RBD); importantly the RBD is highly conserved in all PHR proteins but has no previously described function. The RBD of both Pam and RPM-1 are sufficient for binding to rat Rae1, and we have identified specific point mutations in the RBD of Pam and RPM-1 that abolish binding to NPP-17/Rae1.

To address the significance of this interaction *in vivo*, we have performed a series of experiments using the mechanosensory (mec) neurons of *C. elegans*. Previous studies have shown that *rpm-1* loss of function results in defective axon termination in mec neurons leading to axon overgrowth phenotypes, which can be visualized and quantified by expressing GFP specifically in these neurons (3). We have found that transgenic expression of *rpm-1* that is point mutated so that it cannot bind to NPP-17/Rae1 has reduced efficacy in rescuing axon termination defects in *rpm-1* mutants compared to wild type RPM-1. Thus, binding of RPM-1 to NPP-17/Rae1 is required for complete RPM-1 function. Second, we assessed whether loss of function in *npp-17* affects axon termination in mec neurons. Interestingly, *npp-17* loss of function enhances axon termination defects caused by loss of function in *fsn-1*, an F-box protein that mediates RPM-1 ubiquitin ligase activity. This observation is consistent with *fsn-1* and *npp-17* functioning in parallel genetic pathways to mediate *rpm-1* function. Thus, our study reveals a critical postmitotic role for Rae1 and a novel mechanism by which PHR proteins function in neuronal development.

1) Nakata et al., Cell 2005 2) Grill et al., Neuron 2007 3) Schaefer et al., Neuron 2000

ANALYSIS OF THE SPATIOTEMPORAL EXPRESSION OF MICALS DURING MOUSE CEREBELLAR DEVELOPMENT

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Semaphorins and plexins have been shown to be important for the proper development of the cerebellum, particularly during the migration of the cerebellar granule cells. In *Drosophila*, Molecule Interacting with CasL (MICAL) facilitates semaphorin-plexin-A mediated signaling in neurons. It is unknown, however, whether MICAL also contributes to the proper development of the vertebrate nervous system. Unlike *Drosophila*, vertebrate species have three different MICAL proteins, MICAL-1, MICAL-2 and MICAL-3. The aim of this study was to investigate the expression pattern of the three different vertebrate MICALs during postnatal cerebellar development, which corresponds to the time of cerebellar granule cell migration, using *in situ* hybridization. We show that all three MICALs are expressed in the cerebellum during the examined time window (P0-P15). Interestingly, MICALs show a partly distinct expression pattern. All MICALs are expressed throughout both internal (IGL) and external granule cell layer (EGL). However, MICAL-3 shows a more robust expression in the EGL compared to the IGL. This difference in expression intensity between the IGL and the EGL is not observed for MICAL-1 and MICAL-2. Moreover, MICAL-1 is expressed in the inner part of the EGL (iEGL), while MICAL-2 and -3 show highest expression in the outer EGL (oEGL). MICAL-1 and MICAL-3 are also expressed by Purkinje cells, while MICAL-2 is not. Finally, MICAL-1 is expressed by most cells in the deep cerebellar nuclei, while MICAL-2 expression is very low in these cells. These distinct expression patterns suggest that vertebrate MICALs could play important and distinct roles during postnatal cerebellar development. Future experiments will address the functional role of MICAL proteins in the migration and axon guidance of different cell types in the cerebellum.

A NOVEL IGSF PROTEIN REGULATES AXON PRUNING OF MUSHROOM BODY γ NEURONS

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Neural remodeling by axon pruning is widely used during the development of neural circuits throughout evolution. Understanding the molecular mechanisms regulating developmental axon pruning should provide a broad insight into the mechanisms of axon fragmentation and elimination during development, disease and after injury. Developmental remodeling of axon pruning has been observed in nervous systems ranging from nematodes to mammals, yet the molecular mechanisms regulating axon pruning remain mostly unknown. In particular, little is known about how axons receive extracellular signals that trigger their degeneration.

Drosophila melanogaster mushroom body (MB) γ-neurons is an excellent model to investigate the molecular mechanisms of axon pruning due to its stereotypy and wide spectrum of genetic tools at hand. A MARCM forward genetic screen identified a novel immunoglobulin super family (IgSF) protein that we named Plum, as a key protein involved in initiation of axon pruning in a cell-autonomous manner. By performing detailed structure function analyses we identified a sub-segment of the extracellular domain of Plum, which is both necessary and sufficient for the initiation of axon pruning. In contrast, we demonstrated that the cytoplasmic domain is not required for Plums function in axon pruning. *In-vivo* mosaic gain of function experiments, combined with *in-vitro* aggregation assays suggest that Plum binds a heterophilic ligand that is present in limited amount. Taken together, our observations indicate that plum functions as a co-receptor leading to the initiation of axon pruning.

PRESENILIN-DEPENDENT RECEPTOR PROCESSING IS REQUIRED FOR AXON GUIDANCE

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The Alzheimer's disease-linked gene presenilin is required for intramembrane proteolysis of APP, contributing to the pathogenesis of neurodegeneration that is characterized by loss of neuronal connections, but the role of Presenilin in establishing neuronal connections is less clear. Through a forward genetic screen in mice for recessive genes affecting motor neurons, we identified the Columbus allele, which disrupts motor axon projections from the spinal cord. We mapped this mutation to the Presenilin-1 gene. Motor neurons and commissural interneurons in Columbus mutants lacking Presenilin-1 acquire an inappropriate attraction to Netrin produced by floor plate due to an accumulation of DCC receptor fragments within the membrane that are insensitive to Slit/Robo silencing. Our findings reveal that Presenilin-dependent DCC receptor processing coordinates the interplay between Netrin/DCC and Slit/Robo signaling. Thus, Presenilin is a key neural circuit-builder that gates the spatiotemporal pattern of guidance signaling, thereby ensuring neural projections occur with high fidelity.

NOTCH SIGNALING INHIBITS AXON REGENERATION

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Damaged neurons often fail to regenerate their axons. We show that Notch signaling functions in mature *C. elegans* neurons to inhibit regeneration. Notch signaling occurs when the transmembrane Notch protein is cleaved by an ADAM metalloprotease and by gamma-secretase. These sequential cleavages liberate and activate the Notch intracellular domain (NICD). We find that mutant animals lacking Notch (*lin-12*), ADAM10 (*sup-17*), or the catalytic component of gamma-secretase (*sel-12* and *hop-1*) have increased regeneration. Conversely, animals that overexpress these genes, or that carry gain-of-function alleles, have reduced regeneration. Thus, Notch is a potent inhibitor of regeneration.

Notch pathway components are expressed in a variety of tissues, including post-mitotic neurons. We find that Notch functions cell-autonomously to inhibit regeneration, as expression in neurons is necessary and sufficient to inhibit regeneration. Neuronal Notch signaling likely acts via a transcriptional mechanism to inhibit regeneration, as an mCherry-tagged NICD is localized to neuronal nuclei and is sufficient to inhibit regeneration. These data define a novel function for neuronal Notch signaling in inhibiting axon regeneration.

Molecular pathways that inhibit axon regeneration are potential targets for therapy after nerve damage or disease. To determine when Notch signaling acts to inhibit regeneration, we blocked Notch signaling at the time of injury using a conditional allele of ADAM10/*sup-17*. We find that Notch signaling is required *after* injury to inhibit regeneration. Next, we injured neurons in wild-type animals and then injected these animals with a small-molecule inhibitor of gamma-secretase. This post-injury treatment improves regeneration. Thus, post-injury inhibition of Notch signaling helps improve regenerative outcomes after nerve injuries.

LAR RECEPTORS AND HSPGS ARE REQUIRED FOR PERIPHERAL SENSORY AXON INNERVATION OF THE SKIN

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Trigeminal and Rohon-Beard (RB) neurons sense somatosensory stimuli in zebrafish larvae. During development, these neurons extend peripheral axons that arborize as free endings within the skin, but how these axons are guided to the periphery is not known.

We conducted an *in situ* hybridization screen for cell surface receptor proteins expressed in somatosensory neurons during development. Four protein tyrosine phosphatase receptors (PTPRs) (LAR, LAR2, PTPRD, and PTPRN2L) were notably enriched in trigeminal and RB neurons. Of the four PTPRs, LAR, LAR2, and PTPRD belong to the LAR subfamily. Morpholino knock down of individual LAR family genes caused no obvious defects, suggesting that they may function redundantly. We therefore created a putative LAR2-dominant negative (DN) transgene comprising the extracellular and transmembrane domains, but lacking the intracellular phosphatase domains. Overexpression of LAR2-DN in somatosensory neurons disrupted peripheral axon guidance to the skin: some LAR2-DN-expressing axons wrapped around muscles, while others wandered beneath the skin. We next constructed LAR-DN, PTPRD-DN, PTPRN2L-DN and DSCAML-DN transgenes. Overexpression of LAR-DN resulted in strong skin innervation defects (similar to LAR2-DN), but PTPRD-DN produced only mild defects, and neither PTPRN2L-DN nor DSCAML-DN expression caused innervation defects. Furthermore, simultaneously knocking down LAR and LAR2 with morpholinos phenocopied LAR-DN and LAR2-DN defects. Taken together, these data demonstrate that LAR receptors are required in neurons for peripheral axon innervation of the skin, and that LAR and LAR2 function redundantly in this process.

Heparan and chondroitin sulfate proteoglycans (HSPGs and CSPGs) are ligands for LAR proteins in other contexts. To test whether these molecules are involved in peripheral axon pathfinding, we examined *dackel* mutants, which have a lesion in a glycosyltransferase implicated in heparan sulfate biosynthesis. *dackel* fish display peripheral axon skin innervation defects similar to the LAR- and LAR2-DN phenotypes. To test whether HSPGs function as axon attractants, we locally degraded them by directly injecting heparinase into live larvae. Sensory neuron peripheral axons avoided heparinase-, but not chondroitinase-injected, areas of skin, consistent with an attractive role for HSPGs. Finally, mutating LAR2-DN to disrupt HSPG binding reduced its ability to disrupt axon guidance. We hypothesize that HSPGs act through axonal LAR receptors to guide them to skin. Further characterization of the LAR-HSPG interaction is ongoing.

DEGENERATING LARVAL AXONS SECRETE SEMAPHORIN-2A AND -2B TO PATTERN DENDRITES OF OLFACTORY PROJECTION NEURONS

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The Drosophila olfactory system is an excellent model to study how neural circuits develop their complex and yet precise connectivity. Each projection neuron (PN) targets its dendrites to one of ~50 glomeruli in the fly antennal lobe. Previously we have shown that the transmembrane Semaphorin, Sema-1a, contributes to the precision of these connections. Levels of Sema-1a cell autonomously specify dendrite position along the dorsolateral-ventromedial axis of the antennal lobe (Komiyama et al., Cell 128, 399-410, 2007). What is the ligand for the Sema-1a receptor that initially confers this directionality? Here, we show that the secreted Semaphorins, Sema-2a and -2b, are released by the degenerating larval antennal lobe ventromedial to the developing adult antennal lobe. They repel dendrites of PNs expressing high-levels of Sema-1a to the dorsolateral adult antennal lobe. In flies double mutant for sema-2a and -2b, dorsolateral PNs mistarget their dendrites ventromedially. This mistargeting in sema-2a,2b mutants can be rescued by overexpression of Sema-2a in larval ORNs. Could the secreted Sema-2a and -2b be the ligands for Sema-1a? In support of this hypothesis, we found that Sema-1a and the secreted Sema-2s have opposing expression patterns, bind, and genetically interact. We conclude that Sema-2a and -2b secreted from larval ORNs provide a repulsive ligand for Sema-1a-expressing PNs to instruct their targeting along the dorsolateral-ventromedial axis in the developing antennal lobe. In this way, a degenerating brain structure can instruct the wiring of developing neural circuits.

THE CORE APOPTOTIC EXECUTIONER PROTEINS CED-3 AND CED-4 PROMOTE NEURONAL REGENERATION IN *CAENORHABDITIS ELEGANS*

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How neurons in their native environments respond to, and recover from, localized physical damage is poorly understood and of great relevance to medical research. Femtosecond laser surgery allows precise cutting of individual axons within living *C. elegans*, such that *in vivo* regeneration can be directly observed. We are applying this technology to investigate the role of the cell death machinery in the neuronal response to traumatic damage. We have found that CED-3 caspase, extensively characterized for its role as the essential core executioner protease in apoptosis, acts to promote early events in axonal regeneration. Time-lapse imaging reveals mutant defects in the early stage of regenerative growth cone formation, *i.e.*, the sprouting of short, often transient, exploratory processes. Apoptotic caspase activator CED-4/Apaf1 is also required for efficient regeneration, but the upstream apoptotic regulators CED-9/Bcl2 and EGL-1 and CED-13 BH3 domain proteins are dispensable, revealing regulation mechanistically distinct from *C. elegans* apoptosis. Regeneration also depends on caspase *csp-1*, as well as caspase regulators *csp-2* and *csp-3*. Finally, we have found that the Ca^{2+}-storing endoplasmic reticulum chaperone calreticulin *crt-1* is necessary for efficient regeneration and appears to contribute to damage induced intracellular calcium signaling, acting upstream of CED-3 in response to nerve damage. This study reveals an unexpected reconstructive role for proteins known to orchestrate cell death.

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DECIPHERING MOLECULAR SIGNALING INVOLVED IN THE INNERVATION OF ARTERIES

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The vascular and nervous systems share several anatomical and functional parallels. Both systems use a complex branching network of nerve cells or blood vessels to penetrate all regions of the body and provide a bidirectional flow of information. The establishment of a precisely wired network requires that correct connections are formed between developing nerves or vessels and is achieved through an ordered series of guidance decisions. As a result, some nerves and vessels are locally found to be aligned in the body as they seem to follow the same route, and arteries are innervated by sympathetic axons to regulate vascular tone whereas nerves are surrounded by a vascular plexus to provide oxygen and nutrients.
Indeed, sympathetic axons have been shown to follow arteries to navigate toward their target, but arteries are also a target for sympathetic innervation. We are interested in understanding how the innervation of arteries occurs during mouse development, under normal or pathological circumstances, and which molecular signals are involved in sympathetic axon guidance.
In this study, we have been investigating the innervation of arteries at several stages of development and have identified different steps in this process. We also performed a microarray analysis of arteries before and after innervation in order to identify a wide range of molecules that could be governing the innervation of arteries. We identified secreted and membrane bound molecules that are expressed by smooth muscle cells and endothelial cells of arteries and that are regulated upon innervation. We then tested the effect of those molecules *in vitro* and found that some had a repellent effect since they induced a collapse of sympathetic growth cones, and some affected the branching of axons. In addition, mice inactivated for such molecules displayed impared innervation.
Taken together, our results suggest that several molecules can affect arterial innervation and have a different effect on sympathetic axons. This finding reflects the fact that the innervation of arteries is a complex process that require axon guidance, branching and stabilisation of neurovascular junctions.

AGE-DEPENDENT CHANGES IN SYNAPTIC CONNECTIVITY DURING NEURODEGENERATION

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Adult-onset neurodegenerative diseases are characterized by the age-dependent loss of synaptic connectivity, which is considered a critical event during disease pathogenesis. To directly investigate the effects of aging on synaptic connectivity we investigated the effects of dietary restriction (DR), a manipulation the extends lifespan in many species, on synaptic morphology and neurotransmitter release at the NMJs innervating the CM9 muscle group located on the adult Drosophila proboscis in dynactin complex mutants (*DNGlued*), a model of motor disease . We find that both total synaptic innervation and quantal release of neurotransmitter are significantly reduced in *DNGlued* mutants with increasing age compared to controls. Extension of the lifespan of mutant flies by dietary restriction (DR) significantly reduces both of these declines. Analysis of proboscis extension velocities in young *DNGlued* mutant flies reveals a significant decline in motor function between 7 and 21 days that is improved by DR. Analysis of total synaptic innervation and neurotransmission in 21-day-old DR flies finds no gross differences in synaptic innervation versus control flies, but large increases in quantal release. These data provide evidence that reduced presynaptic function is an early event during the pathogenesis of neurodegeneration in dynactin complex mutants, and that the ameliorating effects of DR on motor function are the result of improved neurotransmission.

AXON GROWTH INHIBITION BY MYELIN: NGR1, RELATED PROTEINS AND PIRB.

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A spectrum of axonal tracts grow in NgR1-/- mice or when NgR-Fc decoy is infused after CNS injury. In addition to NgR1, both PirB and NgR2 have been implicated as myelin inhibitor receptors in various in vitro studies. In vitro assessments of Nogo-A and myelin action have consistently shown that NgR1 is necessary for acute growth cone collapsing activity of Nogo-66 or myelin. However, in certain cell types, chronic outgrowth inhibition by these reagents occurs in NgR1-/- samples and may be mediated by PirB. Nogo-A has three domains with high affinity for NgR1 separated by hydrophobic segments. The most relevant reagent will contain all 3 domains, not just one. Therefore, we developed a method to express and purify in detergent a 22 kDa fragment of Nogo-A with all 3 segments. This molecule is >10-fold more potent than Nogo-66 in DRG growth cone collapse assays. To study neurons relevant for spinal cord injury, we cultured neurons from cerebral cortex. When embryonic neurons are first plated, they express little or no NgR1, but high levels are detected by 3 weeks in culture. At this time point, the cultures are scraped with a custom-engineered multi-pin tool in 96-well plates to create reproducible axonal damage. Fibers then regenerate into the lesion over the next 5 days. Regenerating fibers have growth cones which are collapsed by Nogo-22 kDa more strongly than by Nogo-66. When soluble Nogo-22 kDa is added throughout the 5-day incubation period, regeneration is inhibited. Inhibition of regeneration from relevant neurons by a relevant ligand is abolished in NgR1-/- cultures, documenting that NgR1 plays a role in limiting regeneration.

It is possible that PirB, NgR1, NgR2 and potentially NgR3 play redundant roles in the limiting adult CNS axon growth. We have generated double and triple mutant mice and are now breeding quadruple mutants. The double and triple mutants show no obvious phenotypic abnormality of development. For in vivo studies, the optic nerve provides a homogenous unidirectional set of fibers accessible to crush injury. We have examined the single NgR1 deletion to provide focused assessment of regeneration per se. Previous work showed that dominant negative NgR1 produced a mild degree of regeneration, but synergized with a zymosan injection that enhances intrinsic growth state. NgR1 deletion blocks the pathway more fully, and we have now found that NgR1-/- mice show substantial optic nerve regeneration, which is enhanced by zymosan injection. Thus, NgR1 has a direct role in limiting adult CNS regeneration. The genetic roles of NgR2 and NgR3 and PirB in this regeneration model will be reported.

VANGL2 MEDIATES WNT/PLANAR CELL POLARITY SIGNALING BY ANTAGONIZING DVL1-INDUCED FRIZZLED3 PHOSPHOSPHORYLATION AND PROMOTING FRIZZLED3 ENDOCYTOSIS IN COMMISSURAL AXON GROWTH CONE GUIDANCE

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The Wnt family proteins play crucial roles in axon guidance. We previously showed that Wnt-Frizzled signaling controls anterior turning of spinal commissural axons after midline crossing (Lyuksyutova et al., 2003) and that components of planar cell polarity (PCP) pathway are required in Wnt-guided axon turning. Here, we report that Dvl1 and Vangl2 regulate the Frizzled3 cell surface level on the plasma membrane in opposite ways, with Dvl1 increasing cell surface Frizzled3 level and Vangl2 decreasing it. Further more, Disheveled induces Frizzled3 hyperphosphorylation and hyperphosphorylated Frizzled3 accumulates at the plasma membrane, resulting in reduction of PCP signaling. Vangl2 antagonizes this Dvl1-induced Fzd3 phosphorylation and membrane accumulation, promoting PCP signaling. Moreover, our data suggest that GRK2 is a potential kinase for Frizzled3 hyperphosphorylation. Next, we examined the mechanism of Frizzled3 trafficking. We found that small GTPase Arf6 is colocalized with and regulates Frizzled3 levels and PCP signaling activity. We found that upon Wnt protein addition endogenous Frizzled3 and Vangl2 become concentrated to the growth cone. Using spinning disk confocal live imaging, we found that Vangl2 protein is localized to the tips of the filopodia which are growing longer but not to the tips of the filopodia that are shortening and Frizzled3 and Vangl2 proteins rapidly relocalize within the growth cone upon Wnt protein addition (within minutes). Because both Frizzled3 and Vangl2 can target Dvl1 to the growth cone plasma membrane, we propose that Vangl2 promotes PCP signaling locally in growth cone filopodia tips by competing Dvl1 away from Frizzled3 to allow Frizzled3 to be internalized, probably regulated by Arf6, and causes anterior turning of post-crossing commissural axons in the Wnt gradient. Lyuksyutova, A.I., Lu, C.C., Milanesio, N., King, L.A., Guo, N., Wang, Y., Nathans, J., Tessier-Lavigne, M., and Zou, Y. (2003). Anterior-posterior guidance of commissural axons by Wnt-frizzled signaling. Science 302, 1984-1988. * These two authors contributed equally

CONVERTING DSCAM1 FROM HOMOPHILIC TO HETEROPHILIC SPECIFICITY INACTIVATES SELF-AVOIDANCE IN VIVO

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Dscam proteins are single pass type I transmembrane domain containing proteins of the immunoglobulin superfamily. Alternative splicing of Drosophila Dscam1 potentially generates 19,008 closely related ectodomains, which exhibit isoform-specific homophilic binding. Genetic and molecular studies suggested a model in which the repertoire of Dscam1 isoforms provides each neuron with a unique identity by which neurons can distinguish their self neurites from those of the other neurons. Self neurites bind to each other and are subsequently repelled. This self-avoidance is required for axon branch segregation, uniform coverage of dendritic receptive fields and synaptic specificity. That Dscam1 isoforms mediate homophilic recognition was hypothesized to be a central feature of its role in self-avoidance. However, this has not been shown in vivo. Here, we critically assess the role of Dscam1 mediated homophilic binding in vivo. Using X-ray crystal structures as a guide, we generated Dscam1 mutant isoforms that have lost the ability to bind homophilically. To ensure that these mutations altered specificity rather than more general structural features of the ectodomain, mutations were designed to not only lose homophilic binding, but to simultaneously acquire specific heterophilic recognition. This change in specificity from homophilic to heterophilic binding was demonstrated in biochemical experiments and in vivo. To definitively test whether self-avoidance relies on homophilic repulsion, we introduced homophilic binding deficient isoforms into the endogenous Dscam1 locus using homologous recombination and tested their function in different developmental contexts. These studies demonstrated that Dscam homophilic interaction is essential for self-avoidance in both axons and dendrites.

SPATIALLY AND TEMPORALLY CONTROLLED MAMMALIAN DSCAM ISOFORMS ORCHESTRATE CIRCUITRY FORMATION IN CNS

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Down Syndrome cell adhesion molecule (DSCAM) is a member of the Ig superfamily that participates in numerous developmental processes in both vertebrates and invertebrates. In Drosophila, Dscam can potentially generate up to 19008 isoforms in the ectodomain that allows for it to mediate highly specific self-recognition. In contrast, mammalian DSCAM has been long thought of as the product of a single mRNA, lacking the alternative splicing its Drosophila counterpart undergoes. Perplexingly, DSCAM maintains similar properties in Drosophila, chick and mouse, with it serving as both as an attractive Netrin-1 receptor and as a homophilic receptor mediating repulsion.

We previously showed that eliminating DSCAM in the mouse spinal cord by RNAi inhibits commissural axon growth and turning. To further analyze the contribution of DSCAM to midline guidance, we analyzed a line of DSCAM mutant mice ($Dscam^{del17}$). The $Dscam^{del17}$ mice exhibit severely compromised mosaic tiling and neurite arborization in the retina. Surprisingly, our analysis of commissural neurons by TAG-1 immunofluorscence did not reveal any phenotype. Analysis of other regions, such as the colliculus and cerebellum, also gave contradictory results. The colliculus, like the retina, exhibits dramatic defects, displaying fasciculated and mistargeted of retinocollicular projection axons, while the cerebellum, like the spinal cord, did not have any obvious phenotype, showing normal Purkinje cells, which highly express DSCAM.

These findings raise the question: Why is there such range in the phenotypes in different parts of the CNS of this mutant? One possible model is that there are previously unknown DSCAM isoforms involved. To test this we performed biochemical analyses and found that there are multiple detectable isoforms of DSCAM that are distinct in molecular size (~ 240, 260 & 280kDa), developmentally regulated, and differentially expressed throughout the nervous system. We also show that the mutation in the $Dscam^{del17}$ mice only affects the smallest isoform (DS-A) and regions that only express DS-A, such as the colliculus and retina exhibit defects in neurodevelopment, while the spinal cord and cerebellum, which express additional larger DSCAM isoforms that are maintained in the mutant, do not show any discernable phenotypes. Our data suggests that DSCAM is more multi-faceted than previously thought and may mediate diverse functions through distinct isoforms that broaden its homophilic and heterophilic binding repertoire and result in divergent interactions with downstream signaling effectors.

CNP COOPERATES WITH SLIT TO REGULATE DORSAL ROOT GANGLION SENSORY AXON BIFURCATION IN THE SPINAL CORD BY MODULATING THE DYNAMICS OF MICROTUBULE ASSEMBLY

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The C-type natriuretic peptide (CNP) belongs to a family of cardiac- and vascular-derived hormones known for regulating blood pressure and homeostasis of body water via receptor guanylyl cyclases and cyclic guanosine-3',5'-monophosphate (cGMP). We recently found that this hormone also serves as an extracellular cue to regulate axonal development. In culture, addition of CNP promotes axon branching, stimulates outgrowth, and attracts growth cones of sensory neurons from the dorsal root ganglion (DRG). In mice, CNP is required for sensory afferent bifurcation in the developing spinal cord, as DRG neurons in a spontaneous mouse mutant with a mutation in the CNP precursor gene fail to make the second branch. This defect is identical to that found in mice with mutations in the CNP receptor Npr2 or the downstream cGMP-dependnet kinase, revealing a pathway important for regulating axon branching. To understand how CNP regulates bifurcation, we further analyzed the mutant mice and found that it cooperates with Slit signaling and independently regulates different events during bifurcation. Slit appears to guide the primary axon to the dorsal root entry zone during the initial growth, and CNP controls the formation of the daughter branch. This result indicates that guidance and branch formation are tightly associated during bifurcation. This notion is further supported by the role of dynamic microtubules in axon branching of DRG neurons in culture. First, low doses of the microtubule depolymerization drug nocodazole block cGMP-dependent branch formation. Second, cGMP activation leads to dephosphorylation of CRMP2, a microtubule regulator, which in turn increases the polymerization rate and decreases the catastrophe frequency of microtubules. Thus, CNP represents a new class of extracellular molecules that regulate axonal development by cooperating with other cues and modulating cytoskeleton dynamics.

A NOVEL DCC BINDING PROTEIN POTENTIATES NETRIN STIMULATED OUTGROWTH

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The growth and guidance of commissural axons in the developing spinal cord require netrin-mediated activation of deleted in colorectal cancer (DCC). Commissural axon defects are more severe in DCC-/- mice than netrin-1-/- mice, suggesting there are other DCC ligands involved in axon growth. Using the extracellular domain of DCC as a probe for a protein array containing secreted and transmembrane proteins, we found that Cerebellin 4 (CBLN4), a poorly characterized member of the C1q-TNF family, is a DCC-binding protein. Biacore and alkaline phosphatase binding assays verify CBLN4 binds to DCC with a dissociation constant of 3.4 nM. Interestingly, CBLN4 shares molecular properties with "netrin-synergizing activity (NSA)", a factor originally discovered in a side fraction during the purification of netrin-1 that has yet to be purified to homogeneity (Galko et al., JBC 2000). The shared traits of CBLN4 and NSA include their molecular weight, isoelectric point, and the potentiation of netrin-stimulated outgrowth in rat E11 dorsal spinal cord explants. Ongoing analysis of a CBLN4 knock-out mouse will provide insight into the in vivo role of CBLN4 and further our understanding of the mechanisms of axon outgrowth.

"FLYBOW" - A GENETIC APPROACH TO STUDY NEURAL CIRCUIT DEVELOPMENT IN *DROSOPHILA MELANOGASTER*

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The assembly of intricate neural networks during development is essential for the ability of the brain to process information and produce complex behaviors. To facilitate our studies of the molecular mechanisms that direct the formation of specific neuronal connections in the visual system, we have developed a genetic approach, called Flybow (FB), which is based on the mouse "Brainbow" system by Livet et al (2007). Similar to the vertebrate tool, our approach uses stochastic expression of four fluorescent proteins (XFPs). The constructs were designed to be compatible with available *Drosophila* genetic approaches. Encoding sequences of XFPs are arranged in invertible cassettes flanked by opposing FRT sites. Flybow employs a modified FLP/FRT system to induce inversions and excisions of these cassettes, and the Gal4/UAS system to drive expression, thus enabling its use in all tissues. Additionally, the system can be combined with MARCM and RNA interference approaches for loss-of-function analyses. FB variant 1.0 consists of one invertible cassette driving either mCherry or V5-tagged mCerulean expression. FB1.1 contains a second invertible cassette with opposing EGFP and mCitrine cDNAs for expression of four XFPs. Finally, FB 2.0 contains an additional excisable FLP-out cassette flanked by classical FRT sites to refine transgene expression in specific cell types by overlapping Gal4 and FLP activities. We show that FB variants together with different Gal4 drivers active in the visual system can be used to visualize the delicate morphology of neuron and glial subtypes expressing different XFPs with single cell resolution in the same sample.

POSTSYNAPTIC TRANSLATION AND REGULATION OF SYNAPTIC STRENGTH

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During the growth of Drosophila larval neuromuscular junction (NMJ), many molecules and signaling cascades work in concert to ensure that synaptic strength matches the requirements of the rapidly growing postsynaptic muscles. A reduction in postsynaptic receptor activity during larval growth is met by a compensatory retrograde signaling cascade that ultimately culminates in an increase in calcium influx and enhancement of neurotransmitter release. Alterations in postsynaptic receptor expression and activity have been linked to changes in postsynaptic translation in both vertebrate and invertebrate models; however, little is known about the possibility that postsynaptic translation can participate in the regulation of retrograde signaling across synapses. We are interested in testing whether activity-dependent changes in postsynaptic translation can participate in retrograde signaling and homeostatic plasticity. Therefore, we have set out to examine how genetic removal of genes central to translational regulation will affect basal electrophysiological properties at the NMJ and the ability of the synapse to show retrograde homeostatic compensation in response to reduction in postsynaptic activity. Our preliminary data suggest a role for postsynaptic translational regulation down stream of changes in synaptic activity. We are currently testing whether disruption of postsynaptic translation can suppress the homeostatic response at the larval NMJ.

ESSENTIAL ROLE FOR VAV GEFS IN BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)-INDUCED DENDRITIC SPINE AND SYNAPSE PLASTICITY

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Brain-derived neurotrophic factor (BDNF), and its cognate receptor, TrkB, regulate a wide range of cellular processes, including dendritic spine formation and functional synapse plasticity. However, the signaling mechanisms that link BDNF-activated TrkB to actin cytoskeletal remodeling in dendritic spines remain poorly understood. We report here that BDNF/TrkB signaling in neurons activates the Vav family of Rac/RhoA guanine nucleotide exchange factors (GEFs) through a novel TrkB kinase-dependent mechanism. We find that Vav is required for BDNF-stimulated Rac-GTP production in cortical and hippocampal neurons. Moreover, Vav is partially enriched in excitatory synapses in the postnatal hippocampus, but does not appear to be required for normal excitatory synapse density. Rather, we observe significant reductions in both BDNF-induced, rapid dendritic spine head growth and in CA3-CA1 theta burst stimulated (TBS) long-term potentiation (LTP) in Vav-deficient acute hippocampal slices, suggesting that Vav-dependent F-actin remodeling in postsynaptic spines is essential for normal functional synapse plasticity.

ROLE OF MICRORNAS IN AXON DEVELOPMENT

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During development, axons must extend over long distances to establish connections with their targets. Numerous studies have shown that axons can translate proteins, and have identified roles for axonal protein synthesis in axon guidance, but the underlying molecular mechanisms underlying the regulation of axonal mRNA translation are less understood. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by base pairing with mRNAs, and typically initiate mRNA degradation or translational repression. Although recent work has demonstrated the importance of miRNAs in neuron differentiation, proliferation, survival, and dendritic development, the identity and function of miRNAs in axonal development is relatively unknown. To explore the role of miRNAs in axons, we have investigated their functions in the sensory nervous system. We found that conditional knockout of miRNAs in sensory neuron precursors has functional effects on sensory axons in vivo and in vitro. Using a computational approach, we found that loss of miRNAs affects the expression of mRNAs known to be involved in development of the axon. Loss of miRNAs also results in abnormal axon growth rates, cytoskeletal dynamics, and growth cone responsiveness to guidance cues in vitro. We predict that any insight into miRNA functions in sensory neurons may also apply to other neuronal systems to ensure the proper regulation of signals essential for establishment of axonal connections.

SHP2 IS A KEY REGULATOR FOR CONVERTING NETRIN-1 MEDIATED ATTRACTION TO DCC-DEPENDENT UNC5-MEDIATED REPULSION

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Netrin-1 is a bi-functional guidance cue, meaning that it can mediate either attraction or repulsion depending on the cellular and developmental context. Previous studies have identified several families of receptors that can bind to netrin-1. DCC family members mediate attraction; members of the Unc-5 family of receptors mediate repulsion. Co-expression of a member of the Unc5 family of receptors in neurons expressing DCC can convert netrin-mediated attraction to repulsion. In such instances this repulsive response still relies on the presence of DCC, since a DCC-function blocking antibody can eliminate the response. One possible mechanism by which Unc5 proteins could convert DCC-mediated attraction to repulsion is by the recruitment of additional or a distinct set of adapter proteins that can modulate the downstream signaling cascade triggered in the presence of netrin-1.

Our structure-function studies have revealed that tyrosine 581 of Unc5b plays a key role in this conversion of attraction to repulsion. Furthermore, co-precipitation studies show that mutating this residue does not interrupt DCC and Unc5 complex formation following netrin-1 stimulation. This suggests that this mutation disrupts the recruitment of a key signaling molecule required for a repulsive response. Interestingly the Pawson lab has identified the tyrosine phosphatase Shp2 as a binding partner for this conserved tyrosine residue in Unc5 proteins. In a Xenopus turning assay completed with knock-down of Shp2 expression, repulsive responses to netrin were lost, further suggesting that Shp2 plays a key role in converting attraction to repulsion. Since activation of PI3 kinase pathways is known to be involved in attractive responses and Shp2 can influence this pathway, it is likely that the recruitment of Shp2 negatively regulates PI3 kinase activity during repulsion. To test if this response is conserved in vivo, we have started to explore netrin signaling in Shp2 conditional knock out mice. Migrating cerebellar granule cells express Shp2 and are repelled from a source of netrin-1. Our studies have shown that the netrin receptors DCC, Unc5b and Unc5c are indeed expressed by migrating granule neurons. Therefore our current studies aim to examine Shp2 deficient granule neurons for netrin-1 mediated migration defects and confirm Shp2's key role in the conversion of netrin-mediated attraction to Unc5-mediated DCC-dependent repulsion.

ANALYSIS OF AXON DEVELOPMENT FOLLOWING LOSS- OR GAIN-OF-FUNCTION OF FLOTILLIN2, A LIPID RAFT PROTEIN

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Flotillins are intracellular scaffolding proteins associated with the cytoplasmic face of lipid raft microdomains. They co-cluster with GPI-linked proteins, participate in the assembly of intracellular signaling complexes, and have been implicated in multiple signaling events, including Src signaling and cytoskeletal regulation by RhoGTPases. Flotillins are upregulated in neurons after injury and morpholino knockdown of Flotillins inhibits axon regeneration (Munderloh et al, J Neurosci 29:6607-6615). Expression of a truncated form of Flotillin2 designed to be a dominant negative (*flot2ΔN*) caused impaired neurite outgrowth in cultured hippocampal neurons (Langhorst et al, Eur J Cell Bio 87:921-931). However, nothing is known about the functions of Flotillins during normal axon development *in vivo*. We are investigating potential roles of Flotillins during axon guidance in the developing zebrafish embryo. Zebrafish have three Flotillin genes, and of these only two are expressed in neurons – *flotillin1a* (*flot1a*) and *flotillin2* (*flot2*). *Flot1a* expression is restricted to the nervous system, whereas *flot2* is expressed in the nervous system, somites, and head mesoderm. We found that overexpression of full-length *flot2* or *flot2ΔN* causes defects in motor neuron axons, including ectopic spinal cord exit, axon stalling, and abnormal axon branching. This result suggests that proper levels or localization of *flot2* are required for normal axon development. We have also analyzed an insertional mutant for *flot2*. We confirmed that the insertion is a loss of function mutation, as Western blot analysis shows that Flot2 and Flot1a proteins are greatly reduced in *flot2* mutants, and *in situ* hybridization reveals loss of *flot2* transcript. Surprisingly, *flot2* homozygous mutants appear morphologically normal and have normal axon tracts. This observation suggests that *flot2* may not be necessary for axon development *in vivo*, which we will continue to test in future experiments.

NETRIN-4 PROMOTES THALAMOCORTICAL AXON BRANCHING IN A LAMINA-SPECIFIC AND ACTIVITY-DEPENDENT FASHION

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During development, thalamocortical (TC) axons project to the neocortex and form branches to make synaptic contacts with their target cells in layer 4. TC axon branching is thought to be regulated not only by a genetically defined mechanism but also by an activity-dependent mechanism. Our previous study has suggested that neural activity affects the expression of branch-promoting molecules in the target layer (Uesaka et al., 2007). However, it remains unknown what molecule is involved in this process. To address this issue, we attempted to identify a target layer-derived branch-promoting molecule whose expression is regulated by neural activity. A quantitative PCR analysis revealed that expression of netrin-4 in cultured cortical slices was reduced by pharmacological blockade of firing activity or synaptic transmission. The expression pattern of netrin-4 during postnatal developmental stages was studied by using transgenic rats in which lacZ gene was inserted into the netrin-4 gene locus. Netrin-4-expressing cells were observed in layer 4 of the sensory cortices, and the expression level was prominent during the postnatal second week. Axonal labeling with a fluorescent protein in organotypic cocultures of the thalamus and cortex revealed that netrin-4 application markedly increased the number of TC axon branching in the target layer. In contrast, branching was dramatically suppressed in the cocultures of the netrin-4-deficient cortex and wild type thalamus. Furthermore, immunohistochemistry with an antibody against 5-HTT, which is expressed in developing TC axons, demonstrated that the density of TC axon terminals in the somatosensory cortex was significantly lower in the netrin-4 mutants. These results suggest that netrin-4 regulates TC axon branching by being expressed with laminar specificity and activity dependence.

THE DIVERSE ROLES OF RECEPTOR-ACTIVATED SMADS IN BMP-MEDIATED COMMISSURAL AXON GUIDANCE

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Bone Morphogenetic Proteins (BMPs) have disparate functions establishing neural circuitry in the dorsal spinal cord. BMPs first act as a morphogen to induce specific cell fates, including the population of commissural (C) sensory interneurons. Subsequently, the BMPs act as a guidance cue to direct C axons away from the dorsal midline. We are assessing how C neurons interpret the BMPs to accomplish these diverse cellular responses. BMPs regulate cell fate by binding to a complex of BMP receptors (Bmprs) and thereby activating the Smad family of transcriptional regulators. Our studies have shown that the Bmprs also mediate the guidance activities of the BMPs. However, it remains unresolved which intracellular effector is activated by the Bmprs to control axon dynamics. To further understand the role of the canonical BMP signaling pathway in building neural circuitry, we have examined whether the Smad complex also regulates C axon pathfinding.

The BMP specific Smads, receptor-activated (R) Smad1 and 5 are present in the spinal cord during both the period of C cell fate specification and C axiogenesis. However, these Smads have spatially divergent expression patterns suggesting they differentially affect C cell fate and axonal outgrowth. Supporting this hypothesis, preliminary studies of *Smad1* and *Smad5* loss-of-function mutations in mouse embryos suggest that these R-Smads have separable effects on the specification of C neural fate. In contrast, knocking down either Smad protein using RNA interference in chicken embryos during C axiogenesis results in C outgrowth defects. Taken together, these findings suggest that the R-Smads may have both overlapping and distinct roles regulating different cellular processes during C neuronal development.

CELL TYPE-BASED ANALYSIS OF MICRORNA PROFILES IN MOUSE NEOCORTEX AND CEREBELLUM

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Neocortical circuits consist of a rich array of functional units - diverse neuron types with stereotyped location, connectivity patterns, and physiological properties. Neuron identity and phenotypes are largely determined by the unique pattern of gene expression. microRNAs are ~22nt noncoding RNAs that regulate mRNA translation and stability in a sequence specific manner. Recent studies have shown that some miRNAs are expressed in a cell type and/or developmental stage specific manner and may be critical to the establishment and/or maintenance of cell identity. To systematically profile miRNA expression in different type of neuronal cells, we have established a cell type-based “miRNA-tagging” affinity purification system using genetically engineered mice. Cre-LoxP knock-in system is used to express epitope tagged Agonaute 2(tAgo2) protein in specific cell type. Because Ago2 is a core RISC component associating with mature miRNAs, affinity purification of tAgo2 under appropriate conditions co-precipitates bound miRNA from a genetically defined cell type. Deep sequencing is used to profile the captured miRNAs. Using this method, we are profiling miRNAs in major types of neurons in the neocortex and cerebellum. These results will guide functional studies of miRNAs which contribute to cell identity, circuit development, plasticity, and disorder in the neuronal system.

MODERATE MICROTUBULE STABILIZATION REDUCES SCARRING AND ENABLES AXON REGENERATION AFTER SPINAL CORD INJURY

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Hypertrophic scarring and poor intrinsic axon growth capacity constitute major obstacles to spinal cord injury repair. It is known that scarring and axon growth are tightly regulated by microtubule dynamics. We found that moderate microtubule stabilization decreased scar formation after spinal cord injury in rodents via various cellular mechanisms, including dampening of TFG-β signalling. It prevented the accumulation of chondroitin sulfate proteoglycans (CSPGs) and rendered the lesion site permissive for axon regeneration of growth competent sensory neurons. Additionally, microtubule stabilization promoted growth of otherwise poorly regenerating CNS axons of the Raphe-spinal tract. Thus, microtubule stabilization reduces fibrotic scarring and stimulates the capacity of axons to grow. The manipulation of the microtubules may therefore offer the basis for a multi-targeted therapy after spinal cord injury.

ASYMMETRIC PI(3,4,5)P3/AKT SIGNALING MEDIATES AXON PATHFINDING

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The growth cone of developing axons detects gradients of extracellular cues during axonal pathfinding. The action of many chemoattractant cues requires Ca^{2+} influx, but the role of other second messenger systems and how guidance receptor activation regulates plasmalemmal ion channels is largely unknown. Here we report that PI(3,4,5)P3/Akt signaling mediates growth cone guidance by activating plasmalemmal TRP (transient receptor potential) channels. We found that a chemoattractant gradient triggered rapid asymmetric PI(3,4,5)P3 accumulation at the growth cone leading edge, as detected by translocation of a GFP-biosensor. Growth cone chemoattraction required regulated PI(3,4,5)P3 production and activation of the downstream kinase Akt. Genetic perturbation of polarized Akt activity disrupted axon pathfinding of commissural interneurons at the midline in vivo. Furthermore, patch-clamp recording from growth cones revealed that exogenous PI(3,4,5)P3 rapidly activated TRP currents, and asymmetric application of exogenous PI(3,4,5)P3 was sufficient to induced growth cone attraction in a manner that required downstream Ca^{2+} signaling. Thus, asymmetric PI(3,4,5)P3/Akt activation is an early event in the growth cone's detection of chemoattractant cues that links receptor activation to Ca^{2+} signaling and is poised to serve as a central regulator during axon pathfinding.

LOSS OF *SYD-1* FROM R7 NEURONS IN THE *DROSOPHILA* VISUAL SYSTEM CAUSES A LATE PRESYNAPTIC PHENOTYPE DISTINCT FROM THAT CAUSED BY LOSS OF *LIPRIN-ALPHA*

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The RhoGAP-like protein Syd-1 was first identified in *C. elegans* for its role in presynaptic development. We are studying terminal synapse formation in R7 photoreceptors in the *Drosophila* visual system and have identified a null allele of fly *syd-1* in a screen for mutations that disrupt this process. In *C. elegans*, loss of Syd-1 or of the scaffolding protein Liprin-alpha causes similar synaptic defects, and Syd-1 can be rescued by Liprin-alpha overexpression. These results suggest that Syd-1 acts upstream of Liprin-alpha. Consistent with this model, we have found that *syd-1* and *liprin-alpha* mutant R7s share a number of phenotypes. Loss of either *syd-1* or *liprin-alpha* from R7s causes a reduction in terminal bouton size, and a frequent mislocalization of the adult R7 terminal to a layer, M3, at which the R7 growth cone normally only pauses before extending further to its final M6 target. While both the synaptic vesicle marker Syt-GFP and the mitochondrial marker Mito-GFP are normally enriched at R7 terminal boutons, each marker is found instead in puncta distributed along the length of *syd-1* and *liprin-alpha* mutant R7 axons. However, we have also identified a novel presynaptic defect that is specific to loss of *syd-1*: R7 terminals frequently project thin extensions that can branch, extend beyond the normal R7 target layer, and invade the targets of adjacent R7s. These thin extensions typically terminate in small bouton-like structures that contain Syt-GFP and Mito-GFP. While a second, independently-generated *syd-1* null allele also causes this phenotype, we never observe extensions in *liprin-alpha* mutant R7 axons. We examined *syd-1* mutant R7 axons during development and found that, as expected, their decreased bouton size, premature termination, and mislocalized Syt-GFP and Mito-GFP are all evident by 50 hours after puparium formation (hr APF), the time at which R7 axons normally select their synaptic targets. By contrast, we do not observe ectopic extensions until at least 10 hours later. Our preliminary quantifications of the early termination defect at 50 hr APF and in adult suggest that early termination may later be ameliorated by the tendency of *syd-1* mutant R7 axons to sprout extensions. Together, our results suggest that *syd-1* and *liprin-alpha* do not act in a strictly linear pathway, and that R7s can undergo a second, late stage of axon targeting. We are currently testing whether the late extensions in *syd-1* mutant R7s depend on either Liprin-alpha or the downstream active zone protein Bruchpilot.

THE TRANSCRIPTION FACTOR ZIC2 CONTROLS AXONAL LATERALITY AT THE VENTRAL SPINAL CORD MIDLINE

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Most metazoans are bilaterally symmetric and many features of mature neural function depend on the coherent communication between the two brain hemispheres. In order to integrate sensory information from both sides of the body and then elaborate a coordinated response, the nervous system requires both axons crossing at the midline and axons remaining in the ipsilateral side. Therefore, during the development of bilateral circuits there are different classes of axons that at some point of their trajectories have to decide whether or not to cross the midline. The transcription factor Zic2 has been previously described as the determinant of axonal ipsilaterality in the mammalian visual system. Here we show that during the development of vertebrates, the majority of interneurons that arise in the most dorsal domain (dl1) of the spinal cord project ipsilaterally. Zic2 is highly expressed in the dl1 domain and is necessary and sufficient to specify axonal ipsilaterality at the ventral midline through the control of Robo3 expression. These results point to Zic2 as a general determinant of axonal laterality in the nervous system of vertebrates.

AXONAL REGENERATION PROCEEDS THROUGH AXONAL FUSION IN *C. ELEGANS* NEURONS

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Invertebrate axons and those of the mammalian peripheral nervous system are able to regenerate in the adult. Functional recovery takes place when a damaged axon regains connection with its target tissues. In *C. elegans*, following laser axotomy, the regrowing axon still attached to cell body (proximal) is able to reconnect with its separated distal segment through unknown mechanisms. Using the mechanosensory neurons ALM and PLM as a model system, we have found that during axonal regeneration reconnection between the proximal and distal axonal fragments occurs through a mechanism of axonal fusion, with reestablishment of cytoplasmic and membrane continuity. We found that when axonal fusion does not occur the distal fragment inevitably undergoes Wallerian degeneration and the original axonal tract cannot be restored. Through the use of dual colour labeling of adjacent axonal pairs, we found a high level of specific recognition occurring between a proximal re-growing axon and its own separated distal fragment, revealing possible cross talk between the two processes. Finally, from a candidate mutant approach, we have identified a molecule with homology to a human protein implicated in axonal degeneration, as being necessary for successful regeneration and specifically involved in the process of axonal fusion. We anticipate that a similar mechanism of axonal regeneration could be exploited to improve the outcome of axonal regeneration following injury in mammalian systems.

RAPID ENDOCYTIC MEMBRANE RETRIEVAL AND RECYCLING IN NERVE GROWTH CONES

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Growth cones undergo dynamic membrane remodeling during axon pathfinding. Axon outgrowth requires constitutive (tetanus-insensitive) exocytosis, whereas synaptobrevin-dependent (tetanus-sensitive) exocytic machinery is essential for attractive growth cone steering but is dispensable for motility. Repulsive growth cone steering requires clathrin-dependent and -independent endocytic pathways, depending on the repellent cue. Despite these important functional insights, our understanding of membrane retrieval and turnover in the growth cone is rudimentary. We recently devised a live-cell assay using focal application of the lipophilic styryl dye FM 5-95 to demonstrate asymmetric endocytosis across the lateral axis of the growth cone during repulsive steering. Here, we utilize this assay to report the spatial and temporal dynamics of membrane turnover in migrating growth cones. Time-lapse confocal imaging revealed a remarkable rate and unexpected modes of membrane retrieval. These include endocytic hot spots located at the base of filopodia, at the lateral margins of lamellipodia, and along dorsal ridges of the growth cone. Additionally, we observed waves of endocytosis when individual filopodia de-attached and fused atop the growth cone dorsal surface or with other filopodia. To monitor bulk membrane retrieval by macroendocytic events, we focally applied fluorescent dextran during confocal imaging. Bulk endocytic activity positively correlated with the rate of axon extension and required the function of Rho GTPases. Nascent endocytic vesicles containing FM 5-95 or fluorescent dextran moved retrogradely toward the growth cone central domain and often fused with other labeled vesicles within seconds to minutes. Dye-labeled vesicles occasionally unloaded, providing evidence for rapid membrane recycling or transient fusion with the growth cone plasmalemma. Such dynamic endocytic processes may be important for bulk membrane retrieval and for regulating the surface distribution of receptors, ion channels, or other membrane-associated proteins important for growth cone motility and guidance.

RETINAL GANGLION CELL-SPECIFIC RNA-BINDING PROTEIN, HERMES, PLAYS A ROLE IN TOPOGRAPHIC MAP FORMATION

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In the developing visual system, retina ganglion cells (RGC) project in a topographic manner onto the optic tectum. Gradients of Ephrins and their Eph receptors both on the pre- and post- synaptic populations regulate the map formation together with other guidance cues, many of which now have been identified. However, the intracellular signaling response to these cues in the growing axon tip, the growth cone, is poorly understood. Here, we have investigated the role of local translation in map formation, a process known to be important for correct guidance cue response *in vitro*. We focused on RNA-binding proteins, a large class of proteins that transport specific mRNAs to the distal growth cone and regulate their translation. As they can bind hundreds of mRNAs, one of these proteins could potentially regulate the axonal response to multiple guidance cues.
The RNA binding protein Hermes, also known as RBPMS, is a particularly interesting candidate. In the brain, it is expressed exclusively in RGCs, and it is well conserved between several vertebrate species. In *Xenopus lavevis* and *Danio rerio*, Hermes expression begins in RGCs around the onset of topographic map formation, and our previous work has shown Hermes depletion causes defects in axon arborization in the tectum but does not affect guidance through the pathway to the tectum. Here we have used the developing zebrafish visual system as a model to test whether Hermes plays a role in topographic mapping *in vivo*. Knockdown of Hermes by antisense morpholinos caused striking guidance defects along both dorsoventral and anterior-posterior axes of the tectum, with dorsal axons frequently projecting aberrantly into the medial tectum and temporal axons terminating prematurely in the extreme anterior tectum. Furthermore, other guidance defects such as abnormal looping trajectories and axons projecting aberrantly beyond the tectum were frequently observed, with the majority of all embryos showing some kind of guidance defect. Collectively, our data suggest that Hermes function is critical for topographic map formation.

TOWARDS A GENETIC DISSECTION OF GABAERGIC INHIBITORY CIRCUITS IN NEOCORTEX

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In mammalian neocoertex, the delicate balance and dynamic assembly of the functional architecture of cortical circuits are achieved through a rich repertoire of inhibitory control mechanisms. Different forms of cortical inhibition are mediated by diverse yet distinct classes of inhibitory interneurons which signal through GABA transmission at discrete spatial and temporal niches during circuit operation. While the diversity of GABAergic interneurons presents a major obstacle to understanding cortical circuitry, the stereotypy in their physiology and connectivity suggests key contribution of genetic mechanisms in circuit assembly and provides a basis for genetic analysis. However, cell type based genetic tools and strategies are currently scares. Using the Cre/loxp genetic switch, we have generated and characterized ~20 Cre and inducible CreER knockin lines, which reliably targeted major broad types of GABA interneurons. More distinct subtypes are captured by intersection of Cre and Flp lines (e.g. CCK basket cells), and by engaging lineage and birth dating mechanisms (e.g. chandelier cells). These GABA Cre drivers are poised to initiate the first round of genetic dissection of GABAergic circuits in neocortex as well as in other brain regions. Genetic access to distinct cell types will enable a systematic analysis of basic components of GABAergic circuitry. Furthermore, in combination with an expanding set of Cre activated and genetically encoded markers, sensors, transducer, and molecular tags, these GABA drivers will enable a comprehensive analysis of genetically defined cell populations, from their specification, migration, differentiation, to connectivity, physiological action, and role in network dynamics. Genetic targeting thus will allow cell type-based multi-level analysis which promises to link the assembly and function of cortical circuitry. When applied to disease models, GABA driver-enabled multi-level analysis will facilitate understanding the pathogenetic mechanisms of neurodevelopmental and psychiatric disorders.

NPN-1 MEDIATED AXON-AXON INTERACTIONS DIFFERENTIALLY CONTROL SENSORY AND MOTOR INNERVATION OF THE LIMB

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The initiation, execution, and completion of complex locomotor behaviors are depending on precisely integrated neural circuitries consisting of motor pathways that activate muscles in the extremities and sensory afferents that deliver feedback to motoneurons. These projections form in tight temporal and spatial vicinities during development, yet the molecular mechanisms and cues coordinating these processes are not well understood. Using cell-type specific ablation of the axon guidance receptor Neuropilin-1 (Npn-1) in spinal motoneurons or in sensory neurons in the dorsal root ganglia (DRG), we have explored the contribution of this signaling pathway to correct innervation of the limb.

We show here that Npn-1 controls the fasciculation of both projections and that it mediates inter-axonal communication. Removal of Npn-1 from motoneurons leads to severe defasciculation of motor axons in the distal limb and dorsal-ventral pathfinding errors, while outgrowth and fasciculation of sensory trajectories into the limb remain unaffected. Genetic elimination of motoneurons, however, causes thinned and mispatterned sensory trajectories in the developing limb. Deletion of Npn-1 in sensory neurons results in defasciculation of sensory axons and, surprisingly, also of motor axons. In addition, this leads to pronounced defasciculation of both projections following their exit from the spinal cord and before they reach the decision region within the plexus. These findings are corroborated by partial elimination of sensory neurons, which causes defasciculation of motor projections to the limb.

Thus, motor and sensory axons are mutually dependent on each other for the generation of their peripheral trajectories, and we find that they interact in part through Npn-1–mediated fasciculation before and within the plexus region of the limbs.

THE SEMAPHORIN/PLEXIN SIGNALING PROTEIN MICAL IS A NOVEL F-ACTIN DISASSEMBLY FACTOR

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Semaphorins are one of the largest families of axon guidance cues and were characterized based in part on their ability to rapidly disassemble F-actin and "collapse" elongating neuronal growth cones. However, the molecules directly mediating this effect have remained elusive. Recently, we found that a member of a conserved family of multidomain oxidoreductase (Redox) enzymes, the MICALs, associate with cell-surface Semaphorin receptors (Plexins) and are critical for Sema/Plexin-mediated neural connectivity and actin cytoskeletal rearrangements. To better understand the role that MICAL plays in actin cytoskeletal rearrangements we took a biochemical approach and purified a portion of the MICAL protein that is necessary and sufficient for MICAL's effects in vivo. Our results with this purified protein reveal that MICAL directly associates with filamentous actin and thereby provides a conduit between Semaphorin/Plexin and the actin cytoskeleton. To test whether MICAL also directly regulates the organization of actin filaments, we examined MICAL effects on actin polymerization, depolymerization, branching, and bundling using standard biochemical approaches with purified actin. Strikingly, activating purified MICAL with its coenzyme NADPH resulted in specific alterations to the ability of actin to polymerize such that MICAL/NADPH induced the rapid disassembly of F-actin and the inability to reinitiate actin polymerization. Moreover, most actin filaments are organized into bundles in vivo through the action of actin bundling/cross-linking proteins and our results reveal that MICAL also directly disassembled these bundled actin filaments. Electronic microscopic analysis showed that actin filament bundles are much thinner, shorter and less organized in the presence of activated MICAL. MICAL, therefore, is a novel F-actin disassembly factor that provides a molecular conduit through which actin reorganization – a hallmark of cell morphological changes including axon navigation – can be precisely achieved spatiotemporally in response to semaphorins.

HOW TO USE THE PROTEOMIC RESULTS FOR THE GROWTH CONE RESEARCH

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The growth cone is definitely important structure for the neural wiring, synapse formation and axonal regeneration. Continuous rearrangement of cytoskeletons and recruitment of transport vesicles are essential to the growth cone motility, however, it is unclear how the proteins are directly involved in processes of axonal growth. We successfully used a proteomic approach to identify 945 proteins present in developing rat forebrain growth cones, including highly abundant, membrane-associated and actin-associated proteins. We included synaptosome data in our analysis, as the presynaptic axon terminal is the adult counterpart of the growth cone. Approximately 100 proteins were more concentrated in the growth cone than GAP-43, a sole, well-established marker protein of growth cones, using systematic immunocytochemistry based on our proteomic data. RNAi experiments revealed that the reduced expression of 18 species of proteins significantly inhibited the axonal growth. We called all of proteins as “nGAPs, neuronal growth-associated proteins”. Most of these nGAPs we identified have not previously been implicated in axon growth in mammals or in axon pathfinding in well-studied model organisms such as Drosophila or C. elegans, and thus, their identification presents a significant first step forward, providing new marker proteins. Not only these marker proteins, we found some important ones involved in the novel mechanisms of axonal growth among them. In addition, phosphoprotemics is another improtant approach to determine the phosphorylation sites of each protein. We found some important phosphorylation sites of the growth cone proteins. Taken together, the proteomic results are expected to enlarge our knowledge and scope on the growth cone reserach much wider.

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NEW METHODS TO STUDY NERVE BUNDLE ORGANIZATION.

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Recent findings that mammalian nerves are organized within bundles suggested to us that *C. elegans* could be a useful model for understanding how this organization develops[2,3]. We developed approaches to study the *C. elegans* HSN motor neurons, the last neurons to differentiate, extending axons in the L4 stage along an otherwise, fully developed ventral nerve cord (VNC). Thus, the VNC axons that the HSN axons contact in adult animals presumably reflect the path of the HSN growth cones. Until now, only EM analysis of serially sectioned worms provided the resolution to monitor axon positions within nerve bundles[4]. We developed the membrane-localized split-GFP (GRASP)[5] to label the interface between the HSN axons and those of the PVP and PVQ neurons that guide HSN fasciculation in the VNC. These reproduce the fasciculation pattern predicted from EM analysis. Earlier in development, the PVP axon provides a tract for the PVQ growth cone. PVP/PVQ GRASP along the VNC is continuous, differing from the prediction from the EM analysis. This discrepancy has important implications for the use of GRASP and how the VNC develops. We also developed a genetic-based, neuron-specific ablation system to test the roles of specific cells on guidance and bundle organization. Both this and the GRASP techniques are easily adapted for use with other neurons. Finally, we developed an approach to target HSN fasciculation by inducing RNAi after the VNC develops but before the HSN axons extend. We have used this approach to re-evaluate the roles of molecules implicated in HSN fasciculation.

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DEVELOPMENT OF A NEW STRATEGY TO STUDY SEMAPHORIN AND PTEN FUNCTION IN THE NERVOUS SYSTEM

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To study a protein *in vivo* it is desirable to perturb its normal function in a spatially and temporally restricted fashion. We are exploiting a recently developed approach to achieve this, which is based on a strategy that allows protein function to be regulated in a rapid, reversible and tuneable manner[1]. A destabilising domain (DD) is fused to a protein of interest, causing its efficient degradation. Presence of a DD-specific synthetic ligand stabilises the protein and therefore confers biological activity.

To date, DD technology has not been used to study the function of proteins in the nervous system. Here, we combine *in utero* electroporation of constructs expressing DD tagged proteins into the cortex of mouse embryos with subsequent stabilisation by systemic application of the ligand. We have tested methods to deliver the synthetic ligand, analysing its ability to cross the blood-brain barrier, and investigated if DD fusion proteins can be stabilised rapidly and reversibly.

Using this technique we aim to study the interaction of two pathways with multiple roles in brain development, the Semaphorins and PTEN. We have generated DD tagged Semaphorin ligands, DD-PTEN and DD-Cre, which will be used in combination with floxed-PTEN alleles. As the system allows for combinatorial control of two or more DD fusion proteins, it will enable the investigation of possible interactions between these two pathways in a rapid and reversible manner in mice.

[1] Banaszynski LA, Chen LC, Maynard-Smith LA, Ooi AG, Wandless TJ. (2006). A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules
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THE DELETED IN COLORECTAL CANCER (DCC) GUIDANCE RECEPTOR COORDINATES FAST TURNING BEHAVIORS

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In response to guidance cues, the Deleted in Colorectal Cancer (DCC) receptor directs axon and dendrite extension. A major player in guiding neural outgrowth in both invertebrates and vertebrates, the role of DCC is best understood in the guidance of axons to and across the CNS midline. In humans, dominant mutations in DCC have been linked to congenital mirror movement disorders, where the left and right sides of the body involuntarily move in symmetry, rather than in an alternating fashion. A weak *dcc* mutant is viable in mice and these individuals show abnormal left/right limb coordination during walking. However, *dcc* null mutations are perinatal lethal in mice, preventing detailed analysis of the behavioral functions of DCC-mediated axon guidance.

We have identified a zebrafish behavioral mutant, *spaced out (spo)*, in which DCC is disrupted. We have mapped the genetic lesion to a point mutation in a highly conserved residue of the proposed binding domain for the ligand Netrin. *spo* was initially isolated as these mutants perform multiple rapid bends to one side following acoustic stimuli, unlike the strict left-right alternation of movements observed in wild type fish. Mutant fish are viable and motile, allowing analysis of both the cellular and behavioral functions of DCC.

DCC is strongly expressed in subsets of neurons of the spinal cord and hindbrain of larval zebrafish. As the neural circuits regulating motor output are located in these regions, we have focused our initial attention on the guidance of spinal interneurons and hindbrain neurons implicated in motor control. We observe midline crossing defects in a specific subset of identified commissural spinal interneurons and hindbrain neurons, where in *spo/dcc* mutants these neurons extend their axons toward ipsilateral rather than contralateral targets.

To correlate these DCC-dependent circuit elements to their behavioral functions, we record the movement patterns of 5-day old zebrafish larvae in response to a variety of stimuli at millisecond resolution. Using FLOTE software for automated tracking and analysis of the larval movements, we quantitatively compare the kinematic parameters of each step of the movement patterns performed between wild type and *dcc* mutant larvae. While the sensitivity of *spo/dcc* mutant larvae to stimuli is unperturbed, we observe distinct kinematic defects (ex: turning angle, body curvature, duration) when mutant larvae perform separate movement patterns, as well as left-right coordination defects in a variety of motor contexts. We will present our progress in identifying which specific aspects of larval motor behavior are controlled by the identified DCC-dependent neurons.

COLLABORATIVE AND SPECIALIZED FUNCTIONS OF ROBO1 AND ROBO2 IN SPINAL COMMISSURAL AXON GUIDANCE

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Commissural neurons project axons across the floor plate at the spinal cord ventral midline. After crossing, commissural axons turn rostrally, sort into distinct positions within the ventrolateral funiculus, and never reenter the floor plate. Robo1 and Robo2 are receptors for the midline repellents Slit1-3, and upregulation of Robos in post-crossing axons allows expulsion from the floor plate and prevents re-crossing. Before crossing, Robo-mediated repulsion is attenuated by the divergent family member Robo3/Rig-1. To define the relative contributions of Robo family members to commissural axon guidance in mice, we studied commissural axon trajectories in combination mutants between *Robo1*, *Robo2*, and *Robo3*. Our results suggest the existence of another receptor contributing to Slit repulsion since the failure of midline crossing in *Robo3* mutants is rescued largely but not entirely by loss of both *Robo1* and *Robo2*, and since axon guidance defects in mice lacking both *Robo1* and *Robo2* are less severe than in mice lacking all *Slits*. Analysis of post-crossing axon trajectories indicates that Robo1 and Robo2 collaborate to prevent axons from reentering the gray matter and projecting dorsally alongside contralateral pre-crossing axons. We also discovered a previously unappreciated division of labor between Robo1 and Robo2 in post-crossing axons. Robo2 is required for axons to project away from the floor plate into the lateral funiculus. In contrast, Robo1 prevents axonal stalling after crossing. Our results reveal specialized and complementary actions of Robo1 and Robo2 in commissural axon guidance, and suggest the existence of an as yet unidentified Slit receptor.

CALEB IS INVOLVED IN THE FORMATION OF NEURONAL CIRCUITS IN THE CEREBELLUM.

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CALEB, a transmembrane protein is composed of an N-terminal segment that contains chondroitinsulfate chains followed by an acidic stretch, an EGF-like domain, a transmembrane and a cytoplasmic segment. CALEB appears to be generated as a precursor protein that becomes converted in a truncated transmembrane form with an exposed EGF domain. In slices and cultures this conversion occurs at the cell surface which is facilitated by membrane depolarization and Ca^{2+} influx through voltage-gated Ca^{2+}-channels. In the colliculus superior CALEB-deficient synapses displayed higher paired-pulse facilitation, less depression during prolonged repetitive activation, a lower rate of spontaneous postsynaptic currents as well as lower release probability at early but not at mature postnatal stages. These findings indicated that CALEB is essential for maintaining normal release probability at early developmental stages or the measured impairments are the result of developmental deficits and CALEB might be essential for aspects of synapse maturation.
Here, we studied the role of CALEB in the developing cerebellum. In the immature cerebellum CALEB was found to be predominantly expressed on the soma and proximal parts of the dendrites of Purkinje cells, followed by a strong localization in the molecular layer at more mature stages. Furthermore, in immature stages CALEB is highly glycosylated compared to adult stages. This pattern and timing of localization correlates with the period of synapse formation and elimination on Purkinje cells. Therefore, we studied synaptic input to Purkinje cells, first by analysing inhibitory postsynaptic currents. The amplitude of spontaneous IPSC is reduced during early periods, while the amplitude of miniature IPSC was not affected in CALEB deficient neurons. Consistently, the amplitude of evoked IPSCs was found to be reduced, which results in higher paired-pulse facilitation. Analysis of the climbing fiber - Purkinje cell synapse revealed unchanged basic properties of EPSCs, while the maturation of the adult-like, monosynaptic innervations in the CALEB knockout mouse compared to the wild-type appeared at earlier stages. This faster synapse elimination is not induced by an mGluR-mediated signalling pathway triggered by the parallel fibers. These changes in the maturation of synaptic connectivity in the cerebellum lead to deficiencies in the motor coordination of the knockout mouse. In summary, our data show, that CALEB plays a role in the adjustment of neuronal networks of the cerebellum.

MOTOR NEURON CELL BODIES ARE ACTIVELY POSITIONED BY SLIT/ROBO REPULSION

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Many neuron populations migrate long distances. In contrast, motor neuron cell bodies generally appear quite restricted in their migration abilities. Originating within a specific column of progenitors in the ventricular zone, newly born motor neuron cells migrate radially in a single stage to settle in static clusters, the motor nuclei, next to the floor plate. Within these cell clusters, motor neurons receive afferent input and project their axons out of the CNS at specific exit points. Therefore, the position of motor neuron cell bodies is a critical prerequisite for their functional input and output. We propose that motor neurons have the potential to be highly motile cells, and that the location of motor nuclei near the floor plate is set by active positioning via guidance cues.

In the present study, we found that Slit repellents and their Robo receptors play a role in positioning of motor neuron cell bodies during embryonic mouse development. In *Slit* triple mutants or *Robo1/2* double mutants, *Islet1*$^{+}$ motor neurons invaded the floor plate in the hindbrain and spinal cord. Although progenitors first migrated radially as normal, *Islet*$^{+}$ cells then migrated tangentially into the ventral midline, generally just on the ventricular side of the commissural bundle. *Robo1*$^{+/-}$*;2*$^{-/-}$ mutants still have neuronal cell bodies in the floor plate. However, this phenotype was rescued by a single *Robo2* wild type allele, suggesting Robo2 plays a key role in controlling motor neuron migration. Reporter gene staining in *Robo* mutants confirmed that Robo1 and Robo2 are expressed in *lslet1*$^{+}$ motor neurons. To assess the fate of axons from mis-positioned motor neurons, diI tracing was used and revealed that their axons projected longitudinally within the floor plate, and failed to reach their normal exit points. These results suggest that proper positioning of motor neurons is important for their ability to innervate their peripheral targets.

Together, these findings suggest that motor neurons find their normal position in the CNS using repulsive Slit/Robo signals. Slit/Robo signals position motor neurons by restricting their ventral migration. This reveals a novel migration mechanism in which motor neuron cell bodies actively navigate using floor plate guidance cues.

CONTROL OF MOTOR AXON TRAJECTORY BY THE LIM HOMEODOMAIN FACTOR ISL1

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Islet1, a family member of LIM-homeodomain transcription factor, is critical for the acquisition of motor neuron identity however, its roles in subsequent differentiation process is less known. Previously, we demonstrated that a reduction of Islet resulted in a fate conversion from motor neurons to V2a interneurons in a concentration-dependent manner. A part of axon trajectories of these cells ran parallel to the rostrocaudal axis of the spinal cord, exhibiting the characteristics of interneurons. Here we focus on the axon pathways of motor neurons that express relatively lower levels of Isl1, i.e. the *Isl1* hypomorphic cells, but are sufficient to maintain their fates as motor neurons. Without Isl1, the projection of MMC neurons were affected to show defasciculation, midline crossings and other mistargeting defects. A search for the potential downstream target genes under the control of Isl1 is currently under way. Together, our results suggest that Islet1 plays an important role in motor neuron identity as well as their trajectory.

PATHWAY-SPECIFIC GENETIC ABLATION OF GLUTAMATE RELEASE REVEALS A SPECIFIC ROLE FOR SYNAPTIC TRANSMISSION IN VISUAL CIRCUIT REFINEMENT.

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A hallmark of mammalian neural circuit development is the refinement of initially imprecise connections by competitive activity-dependent processes. In the developing visual system retinal ganglion cell (RGC) axons from the two eyes undergo activity-dependent competition for postsynaptic territory in the dorsal lateral geniculate nucleus (dLGN), but the direct contributions of synaptic transmission to this process remain unclear. Here we use a novel genetic approach to prevent glutamate release selectively from ipsilateral-projecting RGCs and find that the release-deficient axons fail to compete-out transmission-competent axons within the dLGN. Surprisingly, however, the release-deficient axons consolidated and maintained their normal amount of dLGN territory in the face of fully active, competing axons. These data demonstrate a specific function for vesicular glutamate release in mediating the exclusion of axons from inappropriate target regions and argue that during CNS circuit refinement certain major components of structural plasticity are transmission-independent.

MOTOR AND DRG AXONS SERVE AS CHOICE-POINTS FOR THE IPSI-LATERAL TURNING OF DI3 AXONS

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The axons of the spinal Intersegmental interneurons are projected longitudinally along various funiculi arrayed along the dorsal ventral axis of the spinal cord. The roof plate and the floor plate have a profound role in patterning their initial axonal trajectory. However, other positional cues may guide the final architecture of interneuron's tracts in the spinal cord. To gain more insight into the organization of specific axonal tracts in the chick spinal cord we focused on the trajectory pattern of a genetically defined interneuron population, dI3 neurons. Utilizing enhancer-intersection between newly characterized enhancer elements, specific labeling of dI3 neurons was attained. dI3 axons are projected ipsi-laterally along two longitudinal fascicules at the ventral-lateral funiculus (VLF) and the dorsal funiculus (DF). dI3 axons change their trajectory plan from the transverse to the longitudinal axis at two novel checkpoints. The axons that elongate at the DF turn at the dorsal root entry zone, along the axons of the DRG neurons, and the axons that elongate at the VLF turn along the axons of motor neurons. Co-labeling of dI1, dI2 and dI3 neurons, exploiting dI1, dI2 and dI3 specific enhancers and different axonal reporters, revealed a population-specific arrangement of the longitudinal axonal fascicules.
To begin to understand the possible transcriptional control of dI3 cell and axonal fate the expression of the Lim-HD gene *Isl1* was manipulated. *Isl1* is expressed in dI3 neurons and in their en passant targets – motor and DRG neurons. The requirement of *Isl1* to the acquisition of dI3 cell fate was studied in an *Isl1* hypomorph mouse mutant that lacks expression of *Isl1* in dI3 neurons, and by ectopic expression in the chick neural tube. The expression of dI3, and other interneurons cell fate markers, was not altered in either the gain or loss of function paradigms. However, Isl1 is sufficient to impose ipsi-lateral turning along the motor axons when expressed ectopically in the commissural interneurons.
The combination of a quick and efficient enhancer element screen in the chick neural tube coupled with cell type-specific gain- and loss-of function of the *Isl1* gene, has deciphered new intermediate guidance cues and a molecular binary switch that controls ipsi versus contra-lateral axonal projection of spinal interneurons.

VEGF MODULATES NMDA RECEPTOR ACTIVITY VIA A SRC-FAMILY-KINASE-DEPENDENT CROSS-TALK

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N-methyl-D-aspartate type glutamate receptors (NMDARs) are best known for their role in synaptogenesis and synaptic plasticity. Much less is known about their developmental role before neurons form synapses. Here, we report that VEGF, which promotes migration of cerebellar granule cells (GCs) during postnatal cerebellar development, does so by modulating NMDARs and enhancing non-synaptic NMDAR-mediated currents and Ca^{2+} influx in these neurons. The VEGF receptor Flk1 forms a complex with the NMDAR subunits NR1 and NR2B and in response to VEGF, formation of Flk1/NR2B co-clusters on the GC cell surface was increased. This VEGF / NMDAR cross-talk relied on activation of Src-family-kinases (SFKs), which increased tyrosine phosphorylation of NR2B. Inhibition of SFKs abolished VEGF-dependent NR2B phosphorylation and amplification of NMDAR-mediated currents and Ca^{2+} influx. These findings identify VEGF as a modulator of non-synaptic NMDARs and highlight a novel link between an activity-independent neuro-vascular guidance cue (VEGF) and an activity-regulated neurotransmitter receptor (NMDAR).

THE TRANSCRIPTIONAL REPRESSOR TRAMTRACK69 IS NECESSARY AND SUFFICIENT TO DOWNREGULATE AXON GROWTH DURING TARGET SELECTION IN THE *DROSOPHILA* VISUAL SYSTEM.

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In vertebrate nervous systems, synapses most commonly occur at axon terminals. Upon reaching their synaptic targets, growth cones lose their motility and become boutons specialized for neurotransmitter release. We are studying terminal synapse formation in R7 photoreceptors in the *Drosophila* visual system. Overlap between R7 axon terminals is prevented by canonical Activin signaling that downregulates the intrinsic motility of R7 growth cones: R7 axons lacking components of the Activin pathway select the correct target layer but extend into the targets of adjacent R7 axons (Ting et al., 2007). In a genetic screen for additional genes that regulate R7 axon targeting, we identified the transcriptional repressor Tramtrack69 (Ttk69) as being required to prevent R7 axons from invading adjacent targets. In addition, *ttk69* mutant R7 axons have multiple filopodia-like protrusions and often branch before reaching their target layer. Ttk69 has an established role in preventing non-neuronal cells from adopting neural fates and is therefore initially absent from R7s. However, we have found that R7s begin to express Ttk69 by 30 hours after puparium formation, just before their axons extend to their final target, and just prior to the first signs of overgrowth by *ttk69* mutant R7s. Premature expression of Ttk69 in R7s prevents their axons from reaching their final target layer but does not affect their neuronal identity. We conclude that Ttk69 is both necessary and sufficient to inhibit R7 axon growth and that the timing of Ttk69 expression is critical to R7 target selection. While much is known about how growth cones respond to external cues, far less is known about how growth cone motility might be regulated by global changes in gene expression. We are currently conducting screens to identify the downstream targets of Ttk69 regulation in R7s. The Activin signaling pathway appears to be one such target as Ttk69 is required for normal accumulation of the Activin effector dSmad2 in R7 nuclei. Ttk69 is also expressed late in the R1-R6 and R8 photoreceptor neurons as well as in dendritic arborization (da) sensory neurons; we are currently analyzing these for evidence of Ttk69-regulated axon or dendrite growth.

RAPID SYNTHESIS OF THE MENTAL RETARDATION PROTEIN OPHN-1 MEDIATES MGLUR-DEPENDENT LTD

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Oligophrenin-1 (OPHN-1) encodes a Rho-GTPase-activating protein (Rho-GAP) whose loss of function has been associated with X-linked mental retardation. We have previously reported that OPHN-1 is involved in regulating synaptic strength at the CA3-CA1 synapse by stabilizing AMPA receptors in a Rho-GAP dependent manner. Here we examined the role of OPHN-1 in long-term synaptic depression (LTD) induced by group I metabotropic glutamate receptors (mGluRs) in hippocampal neurons. Loss of OPHN-1 by RNAi strongly reduced mGluR-dependent LTD. We demonstrate that OPHN-1 directly interacts with Endophilin 2 and 3, proteins involved in AMPAR endocytosis. Using a molecular replacement strategy we found that this interaction is required for mGluR-dependent LTD, but not for basal synaptic strength which is dependent on OPHN-1's Rho-GAP activity. We further found that OPHN-1 is rapidly synthesized upon group I mGluRs stimulation, and that blocking of OPHN-1 synthesis by acute siRNA application suppressed mGluR-dependent LTD. Together these results demonstrate an important role for rapid translation of OPHN-1 in mGluR-dependent LTD in hippocampal CA1 neurons.

DIFFERENTIAL EXPRESSION OF AXON GUIDANCE GENES IN THE PRIMATE MACULA DURING DEVELOPMENT

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Background and aim: The macula is a highly specialized region in primate retina that is characterized by a high density of neural elements, the prevalence of 'midget' circuitry, and the absence of large retinal vessels. These features allow the macula to mediate high spatial acuity and color vision in the central region of the visual field. There are three overlapping stages of development that characterize the morphological specialization of the macula: (1) ganglion cell (GC) axon pathfinding in the retina; (2) definition of the foveal avascular area, and (3) retinotopic mapping onto visual targets. We aimed to identify candidate genes with roles in these different phases. **Methods:** We carried out a microarray analysis, using human fetal RNA at 19-20 weeks' gestation (n=4), to identify genes differentially expressed in the macula and confirmed expression by quantitative RT-PCR and by *in situ* hybridization, using macaque retinas aged between fetal day 55 and adulthood. **Results:** Gradients of mRNA expression in the GC layer were observed for the axon guidance genes EphA6, unc5h4 and netrin G1, which changed over time. EphA6 was highly expressed in the macula during fetal life and levels of expression in the macula increased postnatally. Netrin G1 was highly expressed early in fetal life, but decreased postnatally. Un5h4 was highly expressed in the macula during formation of the avascular area, but was low in early development and postnatally. The anti-angiogenic factors pigment epithelium-derived factor (PEDF) and brain natriuretic protein (BNP) were also highly expressed in the macula during development and postnatally. **Conclusion:** Changing levels of expression of these genes in the macula during pre- and postnatal life suggests they have sequential roles in the three phases of development. The data suggest that EphA6 regulates vascular patterning early in development and characterizes the projection from foveal GC in the postnatal phase. The findings give insight into how the morphological characteristics of the macula may have evolved. All authors contributed equally to these findings.

CHARACTERIZATION OF THE ANKYRIN BINDING MOTIF OF NEUROGLIAN IN SYNAPSE FORMATION

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L1-type cell adhesion molecules (L1-CAMs) are important for proper nervous system development as indicated by mutations in human L1, which result in a variety of neurological disorders including mental retardation, hydrocephalus and spasticity collectively termed as CRASH syndrome. Vertebrate L1 and its homolog in Drosophila, Neuroglian (Nrg) have been well studied in axon pathfinding, neurite extension and cell migration, however, much less is known about their conserved role in synapse formation. Recent studies from our lab revealed that the phosphorylation status of tyrosine in the ankyrin binding FIGQY motif in the intracellular domain of Nrg is crucial for synapse formation of the Giant Fiber (GF) to Tergo Trochanteral Motorneuron (TTMn) synapse in the Giant Fiber Circuit of Drosophila.
The highly conserved FIGQY motif has been known to bind to ankyrin in its unphosphorylated state but to bind to the microtubule stabilizing doublecortin when phosphorylated. More recently it was shown by the Silletti lab that the phosphorylation status also affects the conformation of cytoplasmic domain of L1-CAM and thereby is likely to also affect other interactions at other sites than ankyrin and doublecortin at the FIGQY motif. Different amino acid changes of the tyrosine in the FIGQY motif allow us to affect ankyrin binding and conformational changes independently. In order to separate the two functions in vivo we expressed different nrg mutant constructs in the Giant Fiber Circuit. Here, we present the anatomical and physiological consequences on the GF-TTMn synapse.

NR-CAM, PLEXIN-A1, AND SEMAPHORIN6D MEDIATE THE CONTRALATERAL RETINAL AXON PROJECTION THROUGH THE OPTIC CHIASM

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To establish circuitry for binocular vision, developing retinal ganglion cell (RGC) axons cross or avoid the midline at the optic chiasm. Transcriptional and guidance factor directives are known for the formation of the ipsilateral (uncrossed) RGC projection from the ventrotemporal (VT) retina, but the factors that implement the contralateral (crossed) retinal projection through the optic chiasm are still unclear. The adhesion molecule Nr-CAM is important for midline crossing but only of the late-born RGCs from the VT retina (Williams et al., 2006). Because Semaphorins have been implicated in RGC chiasm crossing in zebrafish, and Ig-CAMs and semaphorin signaling coordinately function in midline crossing in other systems, we explored their role in crossing of RGCs from the non-VT retina and late-developing VT retina at the mouse optic chiasm. Nr-CAM and the transmembrane semaphorin Sema6D are co-expressed in the midline glia at the chiasm midline. Plexin-A1, the receptor for Sema6D, is expressed in crossed RGCs together with Nr-CAM, and in the SSEA-1/CD44 chiasm neurons. Nr-CAM is a novel receptor for Sema6D, and enhances interactions of Sema6D and Plexin-A1. Sema6D (in HEK cells) inhibits crossed RGC outgrowth in vitro, and Nr-CAM and Plexin-A1 together alleviate this inhibition. On chiasm cells and in vivo, RGC crossing requires Sema6D. In support of these data, in Sema6D-/- and Plexin-A1-/-;Nr-CAM-/- mutants, crossed RGC axons defasciculate, misroute into the ventral diencephalon, and more readily project ipsilaterally. Thus, interactions of Sema6D, Nr-CAM and Plexin-A1 in trans and in cis are critical for retinal axon crossing at the mouse optic chiasm. The selective expression of Nr-CAM and Plexin-A1 in crossing RGCs and the coexpression of Nr-CAM and Sema6D along with Plexin-A1 all in the chiasm region point to coordinate interactions that in sum yield transgression of the chiasm midline. Supported by the NIH(NEI) and Uehara Foundation.

THE ROLE OF ODORANT RECEPTORS AND G-PROTEIN SIGNALING IN THE INITIAL TARGETING OF OLFACTORY SENSORY AXONS TO IDENTIFIABLE PROTOGLOMERULI IN THE ZEBRAFISH

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The zebrafish olfactory projection is an attractive system in which to study axonal targeting. It is relatively simple, develops rapidly, and is accessible at all stages of development. Most importantly, sensory axons extend to individually identifiable and spatially invariant condensations of neuropile in the bulb named protoglomeruli. We have obtained several lines of evidence suggesting that a protoglomerulus is innervated by axons expressing more than one type of odorant receptor. First, we detect the expression of 22 odorant receptors in the olfactory epithelium at a time when there are only 12 identifiable protoglomeruli. Second, olfactory neurons transiently expressing constructs in which the E15-1 enhancer element and OR111-7 promoter drive expression of either OR111-7 or RFP project to the same protoglomerulus, suggesting that expression of the OR transgene does not affect targeting. And third, in preliminary experiments that map the connectivity of OR111 family members to the bulb, our results are consistent with them all targeting a single protoglomerulus. These results support a model in which the enhancer present in the transgene is active in a subset of neurons that are destined to project to the central zone protoglomerulus, and this subset contains axons expressing different, possibly related odorant receptors.

We are also investigating the extent to which G-protein mediated signaling is required for protoglomerular targeting. Transgenic OMP:Gal4 fish were mated with fish containing a UAS driven fluorescent marker or the fluorescent marker and a UAS driven dominant negative Gαs/olf construct. In the fluorescence only control fish, axons entered the Dorsal and Central zone, Medial, and LG3 protoglomeruli. Inhibiting Gαs/olf signaling induced axons to enter additional Lateral protoglomeruli that they would normally avoid. Inhibiting Gαs/olf in OR111-7:Gal4 neurons, however, did not alter their projection to the central zone protoglomerulus. These results suggest that this signaling pathway influences the initial targeting of some but not all olfactory sensory axons.

GAD67 LEVELS IN PARVALBUMIN-EXPRESSING INTERNEURONS EFFECTIVELY MODULATE INHIBITORY TRANSMISSION IN MOUSE PREFRONTAL CORTEX

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A highly reproducible molecular pathology in schizophrenia (SZ) is the reduction of mRNA for GAD67, the rate-limiting enzyme for GABA synthesis, in subtypes of cortical inhibitory interneurons, particularly those expressing parvalbumin (PV). PV+ fast-spiking interneurons play an important role in the generation of gamma range oscillations, which are implicated in the dynamic organization of cell assemblies. Dysfunction in PV+ cells is thought to contribute to cognitive deficits in SZ, such as working memory impairment. However, the functional impact of GAD67 reduction on synaptic transmission and network dynamics, a crucial tenet of the GABA hypothesis of SZ, has remained unclear.

To model the cell-type specific reduction of GAD67 observed in SZ, we used the cre/loxp system to conditionally knockdown GAD67 in PV+ cells. Viral-vector mediated GFP expression was used to identify PV+ cells for physiological analysis.

In paired recordings of PV+ to pyramidal cell connections, GAD67 reduction led to faster IPSC decay in two different cortical regions: medial prefrontal cortex (mPFC) and primary visual cortex (V1). Surprisingly, knockdown of one allele of GAD67 led to greater than 50% reduction of IPSC amplitude in mPFC, but resulted in no significant change in amplitude in V1. This region-dependent effect, which appears due to differences in receptor occupancy, indicates that distinct cortical areas can be differentially sensitive to changes in GABA synthesis. Therefore PFC circuitry appears particularly vulnerable to GAD67 deficiency.

in vivo Electrophysiology and behavioral experiments are underway to determine the impact of PV-cell specific GAD67 reduction on the cortical network. Further understanding of the role of GAD67 and PV+ cells in network dynamics will inform future experimental and modeling studies focused on inhibitory interneurons in the pathophysiology of schizophrenia, and may present novel treatment targets.

SPATIAL LOCALIZATION OF G-ACTIN IN GROWTH CONE GUIDANCE

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During axonal guidance, the motile growth cone senses spatiotemporally distributed extracellular signals and then translates into directional steering of the axon through a complex environment to the correct target cells. While the signaling mechanisms underlying distinct guidance molecules are different, the actin cytoskeleton is believed to be the major target of intricate intracellular signaling pathways that leads to specific motile behaviors of the growth cone. Our previous studies showed that directional growth cone responses are mediated partly by local protein synthesis of actin molecules and spatial severing/depolymerization of actin filaments. However, the involvement of globular actin monomers (G-actin) and its implication in actin assembly/disassembly during axonal guidance remain unclear. In this study, we provide evidence to show an unanticipated spatial pattern of G-actin in growth cones of the cultured *Xenopus* spinal neurons, which may play an important role in axonal guidance. First, using various specific probes for monomeric G-actin, we consistently detected a local enrichment of G-actin at the peripheral domain of the growth cone. Notably, the ratiometric analysis showed that the peripheral localization of G-actin was inversely related to that of filamentous actin (F-actin). In live cultured neurons, the differential distribution of G- and F-actin in growth cones was also observed by simultaneous dual-channel imaging of GFP-actin and RFP-Liveact. Importantly, we detected an asymmetric distribution of G-actin across the growth cone in response to the local application of a guidance cue BMP7. Taken together, these results suggest a novel regulation of growth cone guidance by spatially restricting the availability of G-actin for actin polymerization induced by extracellular stimuli.

FUNCTIONS OF ATYPICAL PKC AND THE PAR COMPLEX IN AXONAL GROWTH INHIBITION

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The failure of axon regrowth after spinal cord injury is due to the accumulation of multiple growth inhibitory molecules, including various species of chondroitin sulfate proteoglycans (CSPGs) at the glial scar. The NG2 CSPG is the most abundant CSPGs in the glial scar and is secreted by reactive oligodendrocyte precursor cells. Its accumulation begins as early as 24hrs post-injury and reaches a peak at about 1 week after injury. Previous studies showed that NG2 inhibits axon growth in vitro and that the infusion of anti-NG2 antibodies can promote axon regeneration across the glial scar. Therefore, the accumulation of NG2 may be a significant factor in the failure of axon generation in the CNS.

It is important to understand the intracellular signaling mechanisms by which inhibitory molecules prevent regeneration in order to design more efficient and feasible therapeutics. Although growth-inhibitory molecules can activate Rho GTPases and PKC signaling, little is known about how these two signaling systems are activated and work together.

Using the NG2 CSPG as a model for growth inhibition, we have been studying the role of PKC signaling. Since there are several isoforms of PKC, we first investigated which PKC isoforms may be involved in axon growth inhibition. Treatment of cerebellar granule neurons (CGNs) with a PKCζ pseudo-substrate significantly stimulated axon growth on NG2-coated substrates, whereas either an atypical PKC-specific inhibitor or Gö6976, a conventional PKC-specific inhibitor had only minor effects. To investigate whether NG2 CSPG activates atypical PKC, we assayed the phosphorylation of T410 using immunoblotting and measured the activity of PKCζ with a vitro kinase assay. We found that addition of NG2 causes a rapid and sustained increase in the phosphorylation and activation of PKCζ. NG2 treatment also caused the translocation of PKCζ to the membrane as determined by subcellular fractionation and imaging. Thus by 3 different criteria, the addition of soluble NG2 activates atypical PKC. PKCζ interacts with par6 and par3, forming the Par complex. The functions of the Par complex are dynamically regulated by context-dependent protein-protein interactions. Therefore, we investigated how NG2 regulates the behavior of the Par complex. NG2 treatment increases the association of PKCζ with par6 and decreases the association with par3. In CGNs grown on NG2 substrates, there is diminished axonal par3 and an accumulation of par3 in the cell bodies. Since par3 is linked to KIF3A, this suggests that NG2 may alter or prevent the axonal transport of the Par complex, leading to diminished axonal growth.

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THE CDC42-SELECTIVE GAP RICH REGULATES POSTSYNAPTIC DEVELOPMENT AND RETROGRADE BMP TRANSSYNAPTIC SIGNALING

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Retrograde BMP signaling mediated by the Gbb ligand modulates structural and functional synaptogenesis at the Drosophila neuromuscular junction (NMJ). However, the molecular mechanisms regulating postsynaptic Gbb release are poorly understood. Here we show that Drosophila Rich (dRich), a conserved Cdc42-selective GTPase activating protein (GAP), inhibits the Cdc42-Wsp pathway to stimulate postsynaptic Gbb release. Loss of dRich causes synaptic undergrowth and strongly impairs neurotransmitter release. These presynaptic defects are rescued by targeted postsynaptic expression of wild-type dRich but not a GAP-deficient mutant. dRich inhibits the postsynaptic localization of the Cdc42 effector Wsp, and manifestation of synaptogenesis defects in drich mutants requires Wsp signaling. Importantly, dRich increases Gbb release and elevates presynaptic P-Mad levels. In addition, dRich regulates postsynaptic organization independently of Cdc42. We propose that dRich coordinates the Gbb-dependent modulation of synaptic growth and function with postsynaptic development.

A NOVEL ROLE FOR PROTEIN TYROSINE PHOSPHATASE 69D IN *DROSOPHILA* CENTRAL SYNAPSE FORMATION.

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The functions of many proteins during neuronal development are regulated by a delicate balance of activity between protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). *Drosophila* phosphatase PTP69D is highly expressed in the nervous system and has been well studied in the peripheral nervous system, where it has been shown to be crucial for axon outgrowth, guidance and targeting.
Here we describe the role of PTP69D in the assembly of the Giant Fiber Circuit, which mediates the startle response of the fly to a "light off" stimulus. The circuit consists of paired giant fiber cell bodies localized in the brain that extend a single axon into the second thoracic neuromere synapsing with the tergo trochanteral motorneurons (TTMn), which further innervate the jump muscles. In addition, the giant fibers (GF) connect via the peripheral synapsing interneuron to the dorsal longitudinal motorneurons (DLMns), which innervate the flight muscles. We characterized the anatomical and electrophysiological phenotypes of several PTP alleles that have missense or deletion mutations in the intra- and extracellular domains. We found that the loss of PTP69D disrupted the GF to TTMn synapse. Our data provides evidence that the defects are not caused by errors in guidance or targeting but in synapse formation. Thus our results suggest a novel role for PTP69D in central synapse formation of *Drosophila*.

WNT4 CONTROLS THE FORMATION OF THE MOUSE NEUROMUSCULAR JUNCTION

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Neuromuscular junction (NMJ) formation requires a highly coordinated communication via several reciprocal signaling processes between motoneurons and muscle. Identification of the local and early cues in synaptogenesis is still poorly documented in mammalian NMJ. One of the key pathways of synaptic connectivity is the Wnt signaling. Here, we report that Wnt4 is involved in muscle innervation. Wnt4 expression is regulated during muscle cell differentiation in vitro and muscle development in vivo, being highly expressed when the first synaptic contacts form and downregulated as muscle differentiation occurs. Analysis of the Wnt4 mutant phenotype reveals that muscle prepatterning is present as acetylcholine receptor (AChR) clusters are localized to the central region of the muscle at stage E13.5. However, profound innervation defects are observed in E18.5 mouse mutant embryos with aberrant nerve branching and overgrowth of primary branches bypassing AChR aggregates. AChR cluster distribution is perturbed, the size of AChR clusters is dramatically increased and 30% of AChR clusters are not apposed to nerve terminals. Interestingly, Wnt4 is able to attract developing motor axons in a spinal cord/COS-7 cells coculture assay suggesting a potential role for Wnt4 in axon guidance. Taken together, these data show that Wnt4 acts during the early step of mammalian NMJ formation and suggest a role in axon guidance.

AN INTERACTION BETWEEN NETRIN-1 AND INTEGRIN RECEPTORS IN GROWTH CONES

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The extraordinary ability of growth cones to navigate through a complex environment to their appropriate destinations is required for proper nervous system development. One of the molecules that steers growth cones to their correct targets is the bi-functional axon guidance molecule netrin-1. The ability of netrin-1 to attract or repel growth cones is dependent on several variables, including substrate. Our data show that netrin-1 application causes growth cone collapse of cultured embryonic chick sensory neurons plated on laminin-1 but not on fibronectin, suggesting that laminin-binding integrin receptors may be involved. Integrin receptors are heterodimeric proteins traditionally known to bind extracellular matrix molecules. To test if integrins play a role in netrin-mediated growth cone collapse, we decreased integrin activity with function-blocking antibodies and peptides prior to netrin-1 application. Interestingly, blocking integrin α3 and α6 subunits (known to bind to laminin) decreased netrin-mediated growth cone collapse. However, blocking integrin α4 (not known to bind to laminin) did not decrease netrin-mediated collapse. These results suggest that laminin-binding integrins may be involved in netrin-mediated growth cone collapse. To test for a potential netrin-integrin interaction, we examined if netrin-1 could activate integrin receptors. Integrin activation refers to a conformational change in integrins that leads to an increased ligand affinity state. Netrin-1 increased integrin activation in growth cones, as revealed by immunocytochemical staining with an antibody specific to activated integrins. To further test for a potential netrin-integrin interaction, we performed co-immunoprecipitations (co-IPs) between netrin-1 and integrins α3 and α6 in chick sensory neurons. Preliminary results suggest that netrin-1 can co-IP with these two laminin-binding integrins, indicating a netrin-integrin interaction in sensory neurons. We tested a direct netrin-integrin interaction using affinity columns and found that netrin-1 can bind to a specific integrin α3 peptide. Although netrin-1 has many classic receptors, these data support previous studies that show integrins may also serve as netrin receptors. While published reports reveal a netrin-integrin interaction in two other cell types, our studies are the first to show: 1) that integrins play a role in netrin-mediated growth cone collapse, 2) a netrin-integrin interaction in growth cones and 3) a specific region of integrins can bind to netrin-1.

NETRIN-1 COLLABORATES WITH VIKING-2 TO MODULATE GROWTH CONE REPULSION MEDIATED BY AN UNC5/PUNC HETEROMERIC COMPLEX.

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Netrins are bifunctional, capable of attracting several classes of axons, while repelling others. Netrin-1 attraction is mediated through members of the DCC family, while repulsion is mediated through a receptor complex of DCC and Unc5. Whereas genetic studies in invertebrates and our own research in mice demonstrate that members of the Unc5 family are capable of signaling repulsion independently of DCC, the molecular mechanisms that netrin-1 uses to trigger this response in vertebrates have not been comprehensively explored. In an attempt to identify signaling partners for one of the four Unc5 homologs, Unc5b, we identified the orphan receptor PUNC (Putative Neuronal Cell Adhesion Molecule). PUNC is a member of the Ig superfamily with a short intracellular domain capable of binding to Unc5b, -c and -d, but not to Unc5a or DCC. To explore the contribution of PUNC to Unc5 signaling, we took advantage of a chimeric receptor approach, in which the extracellular domain of Met is fused to the transmembrane and intracellular domain of PUNC (Met-PUNC). The resulting Met-PUNC chimera can be activated by the Met ligand, HGF. In biochemical studies, we found that both receptors, Unc5b and Met-PUNC, need to be activated by their respective ligands to form a heteromeric receptor complex. In addition, we found that heterologous expression of Unc5b and Met-PUNC in Xenopus spinal neurites mediated DCC-independent repulsion in response to a dual netrin-1 and HGF gradient, but not to netrin-1 or HGF alone. These data strongly suggest that a novel extracellular cue is necessary to signal repulsion through a PUNC/Unc5 complex. To identify this missing cue, we screened an expression library and identified a secreted protein, Viking-2, that binds selectively to PUNC, but not to members of the Unc5 or DCC family. We demonstrate that Viking-2, Unc5b/c, PUNC, and netrin-1 mRNA coincide during spinal cord development in vertebrates. Furthermore, Viking-2 and netrin-1 mRNA appear to be expressed in a counter fashion, suggesting that these two cues establish a topographic pattern. We propose that Viking-2 collaborates with netrin-1 to fine-tune DCC-independent repulsion. In vivo, developing axons are surrounded by a changing 3-D environment consisting of multiple guidance cues in various topographical arrangements; the spatiotemporal integration of all the guidance signals ultimately dictates the growth and turning response of axons. Having a dual ligand system that engages and activates a heteromeric receptor complex is an efficient strategy for navigating axons to overcome axial changes and reach their next target.

SLIT1 ENABLES NETRIN1 ATTRACTION OF ROSTRAL THALAMIC AXONS

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A hallmark of the vertebrate brain is ordered topography, where sets of neuronal connections preserve the relative positioning of cells between two structures. The development of this precise pattern of neural connectivity depends on the regulated action of diverse conserved families of guidance cues and their neuronal receptors. Whereas the role of these molecules has been analyzed independently of each other, they are simultaneously encountered by axons in vivo. Here, we show that the combination of two secreted guidance cues controls topography in the thalamocortical projection by distinct unpredictable responses from different populations of axons. Thalamocortical connectivity conveys sensory and motor information to the neocortex and its initial topography is influenced by gradients of guidance cues along their pathway, within the ventral telencephalon. We have previously shown that thalamocortical pathfinding relies on a precise interaction with corridor cells, a small domain of the ventral telencephalon. We now demonstrate that the corridor controls the initial topography of TCAs by performing rostro-caudal flips of the corridor in a specific transgenic mouse line. At the molecular level, corridor cells express gradients of guidance cues along a rostro-caudal pattern, which is in register with those found in the ventral telencephalon, except for the corridor-specific Slit1 expression. Using confrontation assays, ex vivo co-cultures and in vivo studies, we show that intermediate TCAs are topographically positioned by Slit1 repulsion in the corridor. Remarkably, rostral TCAs are positioned towards rostral areas through a novel mechanism in which Slit1 enables Netrin1 attraction. Our study shows that a novel combinatorial function of Slit1/Netrin1 orchestrates thalamocortical connectivity and opens new perspectives on the molecular mechanisms governing brain wiring.

ANTAGONISM BETWEEN THE MT+TIPS MSPS AND CLASP DURING ABL KINASE MEDIATED AXON PATHFINDING IN DROSOPHILA

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Coordination of cytoskeletal dynamics is an essential aspect of accurate growth cone guidance during nervous system development and regeneration. While there is still much to learn regarding how the growth cone integrates multiple guidance cues through signaling pathways to ultimately modulate the cytoskeleton in a localized way, thus driving growth cone forward progression and steering, it is clear that the dynamic microtubules that explore the growth cone periphery with their plus ends play an essential role in this complex process. A key feature of the microtubule plus-ends is a special set of microtubule associated factors, called microtubule plus-end tracking proteins, or MT+TIPs, that track along the ends as they grow. Evidence suggests that the MT+TIP CLASP, downstream of Abelson kinase signaling, may play a key role to affect both microtubule and actin networks during axon guidance, however, the molecular mechanisms by which it functions are still largely mysterious. In order to gain insight into the functional partners of CLASP, we conducted parallel genetic and proteomic screens for CLASP interactors in Drosophila melanogaster. Our screen identified several functional categories of interactors, including cytoskeletal components and signaling proteins. We focused our initial investigation on the MT+TIP Minispindles (Msps, ortholog of Xenopus Xmap215), identified among the cytoskeletal effectors in both genetic and proteomic screens. We demonstrate that Msps functions during axon guidance, and our genetic data in the Drosophila embryonic nervous system suggests a model in which CLASP and Msps converge in an antagonistic balance within the Abl signaling pathway to regulate the growth cone cytoskeletal output downstream of guidance cues. We have now begun to use high-resolution live imaging of cytoskeletal dynamics in Xenopus growth cones in order to determine how the Msps MT+TIP complex responds to changes in guidance cue signaling to affect microtubule dynamics and growth cone behavior.

GENETIC ANALYSIS OF NEURONAL POLARITY IN HIPPOCAMPAL NEURONS

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Neurons are highly polarized cells with a single axon and several dendrites. Cultured hippocampal neurons are widely used as a model system to study the establishment of neuronal polarity. The differentiation of cultured neurons can be subdivided into 5 stages. After 0.5 days in vitro several immature neurites are formed (stage 1-2), which all have the potential to become an axon. At late stage 2, one neurite is selected to become the axon and extends rapidly (stage 3). The establishment of neuronal polarity results in the formation of distinct axonal and dendritic compartments that differ in their cytoskeleton and their protein composition. At stage 4 and 5, dendrite formation and axon maturation occur.
In vitro studies using cultured hippocampal neurons revealed several signaling pathways involved in the establishment of neuronal polarity. The small GTPase Rap1 and the serine/threonine kinases SadA and SadB were identified as essential components which act in different signaling cascades to regulate neuronal development.
In cultured neurons, Rap1B is essential for axon formation. In early stage 2 neurons, localization of Rap1B to the tip of a single neurite determines which neurite becomes the axon. Suppression of Rap1B using RNA interference (RNAi) leads to a complete loss of polarity. The in vivo function of Rap1B and the highly similar Rap1A, which are encoded by two different genes, has not yet been analyzed. To address the physiological function of these GTPases, we analyzed single and double knockout mice for these genes using conditional mutagenesis.
Like Rap1, SadA and SadB are required for neuronal differentiation, but show a different loss-of-function phenotype. Knockout of SadA/B leads to the formation of multiple indetermined neurites, which are positive for axonal as well as for dendritic specific marker. We could show that SadA binds to and phosphorylates the cell cycle checkpoint kinase Wee1 and thereby initiates its degradation. Further analysis of SadA/B double knockout mice revealed differences in the requirement for SADA/B in hippocampal and cortical neurons.

EPH/EPHRIN FORWARD SIGNALING CONTROLS AXON FASCICULATION BY MODULATING MICROTUBULE DYNAMICS

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Within the developing neuromuscular system motor and sensory axons form large bundles that travel along stereotypical routes to reach their target. Both pathfinding and fasciculation processes are highly regulated by a number of interaxonal adhesion and guidance molecules expressed in surrounding tissues. Eph receptors and their ligands the ephrins have been shown to participate in both axon guidance and fasciculation, however, the molecular mechanisms by which they do so remain elusive.
Here we used the sensory-motor system innervating the limb bud as a model and showed that genetic deletion of ephrin-B1 leads to defects in fasciculation of both motor and sensory axons. We further demonstrated using the Cre-lox system that ephrin-B1 acts non autonomously to regulate axonal fasciculation. Using an in vitro culture assay we found that activation of forward signaling induces growth cone collapse and neurite fasciculation. Moreover, we showed that forward signaling regulates microtubule dynamics and we identified a novel effector of the pathway, a microtubule associated protein called ASAP.
Altogether our results suggest that Eph/ephrin signaling regulates guidance but also axon fasciculation by modulating microtubules dynamics with ASAP as an effector.

VEGF REGULATES AXON-SCHWANN CELL INTERACTIONS IN THE PERIPHERAL NERVE

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Vascular endothelial growth factor (VEGF) is a potent angiogenic factor in vitro and in vivo that also affects neuronal development. VEGF is expressed as a combination of 3 major isoforms that are produced by alternative splicing. We have previously shown that the most prevalent of these isoforms, VEGF164, acts on neurons independently of its effects on blood vessels[1]. Previous studies have suggested that VEGF164 is secreted by Schwann cell glia and may also affect these cells[2-4]. We show that, in vivo, VEGF164 is required for axon-Schwann cell (SC) association and SC production during the process of radial sorting in the peripheral nerve. We further found that mice lacking the main neural VEGF164 receptor NRP1 specifically in the neural crest cell lineage also show defective SC-axon interactions. Because NRP1 is not obviously expressed by Schwann cells, but instead on axons, our observations suggest that nerve-derived VEGF signals to developing axons to regulate their interaction with Schwann cells during radial sorting. The discovery of a novel pathway that regulates axon-SC interactions and SC proliferation in peripheral nerves may have implications for future work on PNS regeneration.

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REGULATION OF ADAR2 UNDER EXCITOTOXIC CONDITIONS

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AMPA receptors are glutamate receptors that are tetramers of various combinations of GluR1-4 subunits. AMPA receptors containing GluR1, 3 and 4 are Ca2+ permeable, however, AMPA receptors containing even a single subunit of GluR2 are Ca2+ impermeable. Mostly AMPA receptors are Ca2+ impermeable due to the presence of GluR2. GluR2 confers special properties on AMPA receptors and these properties are due to the presence of arginine at the pore apex; other subunits (GluR1, 3, 4) contain glutamine at the pore apex and allow Ca2+ influx. An RNA editing step, which changes DNA encoded glutamine to arginine, introduces arginine in GluR2. GluR2 RNA editing is carried out by an RNA-dependent adenosine deaminase (ADAR2). Loss of GluR2 editing has been shown to contribute to loss of motor neurons in amyotrophic Lateral Sclerosis (ALS). Relatively higher levels of Ca2+ permeable AMPA receptors have been shown in motor neurons and this has been correlated to lower GluR2 mRNA levels. However, the reason for loss of GluR2 editing is not known. We show that exposure of neurons to excitotoxic levels of glutamate leads to specific cleavage of ADAR2 that may render the enzyme non functional leading to decrease or loss of GluR2 editing which will further result in high Ca2+ influx and excitotoxic death in neurons.

SIGNALLING FROM RAP1B TO CDC42 DURING THE ESTABLISHMENT OF NEURONAL POLARITY

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The polarized morphology of a neuron with a single axon and several dendrites is essential for its function in the nervous system. The early development of cultured neurons can be subdivided into 5 stages. Initially, neurons form several indistinguishable neurites which all have the potential to become the axon (stage 1-2). In late stage 2, one of the neurites is specified as the axon and grows rapidly (stage 3). Dendrite formation and maturation follow in stage 4 and 5, respectively. The specification of the axon is regulated by a signaling cascade that includes PI3K and several GTPases which localize to the tip of the nascent axon and act sequentially to specify axon identity.

The small GTPases Rap1B and Cdc42 act as molecular switches that are required for the establishment of neuronal polarity. Rap1B acts downstream of PI3K and upstream of Cdc42 in the signaling cascade that initiates the establishment of neuronal polarity. Both Rap1B and Cdc42 are essential for axon formation. However, how Rap1B regulates Cdc42 activity is not known so far. It is likely that specific guanine nucleotide exchange factors (GEFs), which act as activators of small GTPases, mediate the activation of Cdc42 by Rap1B. In budding yeast, the Cdc24p GEF links the homologues of mammalian Rap1B and Cdc42 to mediate bud site selection. We investigated a structural homologue of Cdc24p as a candidate for the Cdc42 GEF that links Rap1B and Cdc42 in neurons. Biochemical experiments showed that Rap1B and Cdc42 interact with this GEF. We will present results from overexpression and knockdown experiments in hippocampal neurons to elucidate the function of this GEF. Further experiments will show if Rap1B regulates the function or localization of this GEF.

RETINAL GANGLION CELL AXONS NEED MICRORNA FUNCTION FOR CORRECT PATHFINDING DURING MOUSE VISUAL SYSTEM DEVELOPMENT

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The visual system has been largely investigated to identify the molecular mechanisms controlling the formation of neuronal connectivity during development. In animals with binocular vision, the optic chiasm is a major decision point where retinal ganglion cell (RGC) axons arising from the eyes sort into ipsi- and contralateral projections. In mice, about 97% of the axons cross to the contralateral side, whereas the rest stays ipsilaterally, projecting towards the lateral geniculate nucleus and then into the superior colliculus. Here, RGC axons form connections that obey the rules of a defined topographic map.
In the last years intensive studies in the field highlighted the roles of several polypeptide-encoding genes controlling the different morphogenetic subroutines, which ensure the correct wiring of the visual system. Recently, through the use of conditional inactivation of RNase III enzyme Dicer, we and others demonstrated that microRNAs (miRNAs), a class of small non-coding RNAs, are fundamental regulators of retinal histogenesis and axon guidance decisions at the optic chiasm.
Our results show that mouse embryos with an early loss of Dicer in the retina and in the ventral diencephalon, exhibit a microphthalmia phenotype associated to a high rate of apoptosis during neurogenesis. In these mutant embryos we also noticed a significant increase of ipsilateral projections and defasciculated axons at the optic chiasm and in the retina. Moreover, a considerable number of RGC axons aberrantly project from one eye into the other or enter the diencephalon ectopically. To investigate the possible underlying mechanisms for these severe phenotypes, we first analysed the expression patterns of known key molecules involved in patterning and axon guidance that may have been directly or indirectly affected through the lack of miRNAs in the retina or the chiasm. Using in situ hybridization and immunohistochemistry we find that Dicer mutant retinae are patterned normally along both axes without showing any changes of guidance molecule expression. Similarly, the number and location of Zic2-expressing RGCs is unchanged in Dicer mutant mice, compared to wildtype, suggesting that the specification of the ipsilateral retinal domain is not controlled by miRNAs. Experiments using different conditional Dicer KOs or single miRNA deletions in RGCs are underway in order to define a possible cell-autonomous way-of–action of miRNAs during axon outgrowth. This will help to clarify the molecular pathways involved in RGC axon extension, pathfinding and synapse formation. Our work presents a role for miRNAs as a linchpin for the establishment of the visual circuitry during development.
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ENDOTHELIN SIGNALING IS A CRITICAL GUIDANCE MECHANISM FOR DEVELOPING AXONS

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Blood vessels serve as roadways over which nerves extend as they innervate target fields. Previously, endothelin signaling was identified as a critical vascular-derived axon guidance mechanism that directs axonal growth from the cranial-most sympathetic ganglia, the superior cervical ganglia (SCG), to their appropriate vascular trajectory, the external carotid arteries. Endothelins are small peptide signaling molecules that are most widely known for their hypotensive effects on vascular smooth muscle. There are three endothelins (ET1-3) encoded by distinct genes (*Edn1-3*), which are processed to active forms by two endothelin converting enzymes (Ece1-2), and which act via two G-protein coupled receptors (ET_A and ET_B). Axonal projections from the SCG are attracted primarily by ET3 that is selectively produced by smooth muscle of the external carotid arteries, through activation of ET_A in a subset of SCG neurons.

To address further roles of vascular-derived endothelins in axon guidance, we analyzed mouse embryos carrying mutations in several endothelin ligand or receptor genes and have found many additional examples where endothelin signaling controls specific axon guidance events during embryonic development. These include absence of specific axonal growth along the aortic arch and the superior vena cava from the thoracic sympathetic ganglia (the stellate ganglia, STG) in the cardiovascular system, and absence of specific axonal branching and extension of cutaneous sensory nerves along the radial arteries from the dorsal root ganglia (DRG). In both contexts, the endothelins are produced in vasculature, which accounts for the historically-described neurovascular congruence of the nervous system. Furthermore, we have observed abnormal patterning and projection from the cranial nerves in the absence of endothelin signaling, for which axonal growth and projection are not related to vasculature. Therefore, endothelin signaling plays multiple roles in guiding distinct groups of developing neurons.

NCAM INTERACTS WITH EPHRINA/EPHA TO REGULATE SYNAPTIC DEVELOPMENT OF CORTICAL GABAERGIC INTERNEURONS

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A novel function for EphrinA/EphA repellent signaling through the neural cell adhesion molecule NCAM was identified in development of inhibitory synaptic connectivity in the prefrontal cortex. GABAergic basket interneurons elaborate profusely arborized axons that synapse onto soma of multiple pyramidal neurons, enabling synchronous firing, which underlies working memory. Development of axon arbors and formation of perisomatic synapses between basket interneurons and pyramidal cells was restricted by ephrinA5 treatment in slice cultures of cingulate cortex from wild type (WT) but not NCAM null mutant mice. *In vivo*, genetic deletion of NCAM or ephrinA2/A3/A5 in null mutant mice enhanced axon arborization and increased perisomatic synapses of cortical GABAergic interneurons. NCAM mediated ephrinA-induced axon repellent responses, as shown by the ability of ephrinA5 to induce growth cone collapse through ADAM10 metalloprotease in WT but not NCAM-minus cortical interneurons. NCAM colocalized with EphA3 in processes and perisomatic synapses of basket cells and formed a molecular complex with EphA3 and ADAM10 in postnatal mouse brain.

Surprisingly, ephrinA5 triggered ADAM10-dependent proteolytic cleavage of NCAM, releasing the entire extracellular fragment (NCAM-EC) in mouse neuronal cultures and HEK293 cells. Moreover, NCAM-EC induced growth cone collapse in interneurons, suggesting that cleavage of NCAM is an important mechanistic component in the collapse response. The ability of NCAM-EC to induce growth cone collapse can explain the loss of perisomatic synapses between basket and pyramidal neurons in the cingulate cortex of transgenic mice overexpressing NCAM-EC, a model for elevated NCAM-EC expression in postmortem schizophrenic brain. When tested in a delayed non-match-to-sample task, NCAM-EC transgenic mice were found to have impaired working memory.

These results reveal a new mechanism of synaptic plasticity in which NCAM, ephrinA5/EphA3, and ADAM10 coordinate to limit inhibitory perisomatic innervation of pyramidal neurons, important for appropriate excitatory/inhibitory balance in the prefrontal cortex. By extension, NCAM-interacting molecules (ephrinAs, EphAs, ADAM10) may be candidate targets for mutations associated with cortical GABAergic dysfunction and cognitive deficits in schizophrenia.

REGULATION OF GOLDEN GOAL FUNCTION BY PHOSPHORYLATION

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Golden Goal (Gogo) is a single pass transmembrane protein required for axon guidance of photoreceptors in Drosophila. Gogo is required in R8 photoreceptor axons for the recognition of their temporary target layer M1 and then proceeding to their final destination, M3 layer in the medulla; additionally both extracellular and intracellular parts of Gogo are required for its function (Tomasi et al., 2008). Although the cytoplasmic part does not contain any known domains, we show that its middle third part (C2 fragment) is sufficient for Gogo's cytoplasmic function. We show in vivo a crucial function of a conserved YYD (Tyr-Tyr-Asp) motif within the C2 fragment, as Gogo lacking the YYD motif does not rescue the *gogo*$^{-}$ phenotype. We postulate that the YYD motif is a phosphorylation site and the dephosphorylated Gogo is the functional form involved in R8 targeting, as the non-phosphorylable form of Gogo rescues the *gogo*$^{-}$ mutant phenotype whereas the phospho-mimicking form fails to rescue *gogo*$^{-}$. Moreover, overexpression of constructs mimicking Gogo phosphorylation and Gogo lacking the YYD motif is able to cause stopping of R8 axons at the M1 layer. Thus, we propose that the Gogo phosphorylation status plays a crucial role in the recognition of the M1 layer and proceeding to the M3 layer targeting. We are trying to show the phosphorylation of Gogo *in vivo* using the cell culture system. In our preliminary results we show a genetical interaction between *gogo* and *dinr* (*Drosophila insulin receptor*) suggesting that DInR is a regulator of Gogo phosphorylation status. We will discuss in a more detail the role of DInR in the regulation of Gogo phosphorylation.

MODULATION OF SEMAPHORIN 3A-INDUCED GROWTH CONE REPULSION BY PROTEIN SYNTHESIS IS CONCENTRATION-DEPENDENT.

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Work over the last decade has provided evidence that growth cones require local control of protein synthesis to respond to several axon guidance molecules (Campbell and Holt, 2001; Ming et al, 2002; Wu et al, 2005; Yao et al, 2006). For example, the chemotropic responses to Sema3A *in vitro* are blocked by pharmacological inhibitors of protein synthesis (Campbell and Holt, 2001; Wu et al, 2005; Li et al, 2009). However, a recent study reported that while Sema3A activates translation in growth cones, protein synthesis inhibitors do not block Sema3A collapse, raising questions about the validity of previous findings (Roche et al., 2009).

Here we have sought to understand the discrepancy between these findings. We have shown that chick sensory (DRG) axon growth cone collapse induced by lower concentrations of Sema3A (67.5-375 ng/ml) is prevented by a variety of protein synthesis inhibitors - cycloheximide, anisomycin and rapamycin - but that the extent of this effect falls as the concentration of repellent increases, regardless of the inhibitor used. At higher Sema3A concentrations (≥500 ng/ml) these inhibitors fail to block the collapse response, confirming the observations of Roche et al. (2009), who used comparable concentrations of Sema3A. These Sema3A levels also cause axons to develop a beaded morphology along their length, characteristic of early degeneration, and both growth cones and axons fail to recover normal morphology for more than an hour after Sema3A is removed from the culture medium.

We conclude that growth cone collapse induced by Sema3A at low concentrations is dependent upon mTOR-mediated protein synthesis. This dependence falls at higher concentrations, possibly due to engagement of protein synthesis-independent pathways causing axon toxicity (Ben-Zvi et al, 2008). Activity of these pathways may be triggered by high levels of repulsive signalling, resulting in irreversible growth cone collapse. Collectively, our data demonstrate that protein synthesis-dependent collapse, induced by Sema3A, is concentration-dependent.

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THE LOW AFFINITY NETRIN RECEPTOR UNC5A MEDIATES SHORT-RANGE REPULSION IN VERTEBRATES

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It is well established that netrin-1 is a bifunctional cue and that its effect on axon growth depends on which receptors are expressed on the surface of the growth cone. Genetic studies in invertebrates have demonstrated that netrin-1 can signal repulsion through both DCC-dependent and DCC–independent mechanisms; for example, in *Drosophila*, UNC5 mediates short-range repulsion independently of DCC, while long-range repulsion depends on DCC. Whether these ligand-receptor strategies are conserved in vertebrates is currently unknown. Thus, we investigated whether vertebrates signal repulsion in a DCC-independent manner and which netrin-1 receptor(s) are involved. Using a Xenopus turning assay, we show that spinal neurites undergo a developmental switch from netrin-1 mediated DCC-dependent attraction (stage 20-26) to netrin-1 mediated DCC-independent repulsion (stage 30-36). RT-PCR analysis of Xenopus spinal cords from different stages revealed that upregulation of Unc5a, -b, and -c mRNA expression coincides with the switch to netrin-1 mediated repulsion at stage 32. A combination of molecular and functional assays further revealed that the low-affinity netrin receptor, Unc5a, is the only Unc5 family member capable of signaling netrin-1 mediated repulsion independently of DCC. This repulsion is short range and mediated by the multimerization of Unc5a receptors upon netrin-1 activation. Through domain-swapping experiments, we show that Unc5a multimerization is facilitated by a dual interaction in the intracellular region involving both the conserved death domain and a juxtamembrane region unique to Unc5a that we have termed the homodimerization domain (HD). We also demonstrate that DCC-independent repulsion can be recapitulated with Unc5b receptors when the HD of Unc5a is inserted. Both domains are required for netrin-1 induced Unc5a self-association and DCC-independent repulsion. Our results underscore that Unc5 proteins utilize different domains and take advantage of their distinct netrin affinities in order to mediate either long or short-range DCC-dependent or -independent repulsion. Furthermore, we demonstrate how a family of receptors can mediate a range of responses to the same ligand and suggest that short range DCC-independent repulsion is an economical way to help growth cones navigate through various ligand environments.

THE ROLE OF ROBO3 SUBPOPULATIONS IN THE SPINAL LOCOMOTOR CPG

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Neuronal circuits are basic components of the nervous system. In order to understand how neuronal circuits operate it is necessary to identify the participating neuronal subpopulations and to study the function of the neurons at the molecular level. Spinal cord commissural interneurons (CINs) that project axons contralaterally across the midline are implicated in the coordination of left-right limb movements during locomotion. During development, CINs axons are guided to the floor plate by attractive guidance cues such as Netrin. Upon crossing the midline they switch on repulsive responses to prevent the axons from recrossing. In mammals, the three Slits (Slit1-3) are expressed at the floor plate, and were shown to repel CINs axons through binding to Robo1 and Robo2 receptors. Slit/Robo repulsion is thought to be prevented in precrossing CINs axons by the third roundabout receptor, Robo3, whose expression is turned off after midline crossing. Accordingly, in constitutive *Robo3* knockout mice the commissural axons of the brain fail to cross the midline, suggesting that they could be prematurely repelled by Slits. CINs that are able to revert left-right alternation to strict synchrony have not been defined. We have set out to dissect the contribution of subsets of spinal cord CIN neurons in CPG coordination using a $Robo3^{lox/lox}$ allele. To prevent midline axon crossing from selected subpopulations of CINs, Robo3lox mice were crossed to several transgenic lines expressing Cre recombinase in specific/restricted subsets of spinal cord CINs. Our analysis has shown that deletion of Robo3 results in a complete loss of commissural axons also in the spinal cord. We are now investigating the role of neurons originating from dorsal or ventral subpopulations and will determine the significance of CIN origin in the formation and function of the left-right circuitry in the locomotor CPG.

NMDA RECEPTOR-DEPENDENT MICROTUBULE POLYMERIZATION INTO DENDRITIC SPINES.

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Most excitatory synaptic terminals in the brain impinge on dendritic spines, small actin-rich protrusions that line the dendrites of principle neurons. While spines have historically been thought to be devoid of microtubules (MTs), we and others have shown that MTs polymerize into spines in an activity-dependent manner and can trigger rapid spine enlargement, suggesting that MTs may directly contribute to synaptic remodeling. Here we imaged MTs in live hippocampal pyramidal neurons expressing EGFP-α-tubulin, and induced chemical long-term potentiation (c-LTP) in these cells via acute activation of synaptic NMDA receptors (NMDARs). c-LTP increased the percentage of spines invaded by MTs and the frequency of invasions, with invaded spines displaying prolonged enlargement. Thus, MT entry into spines is NMDAR-dependent and correlates with spine growth.

To determine whether synaptic activity alters MT dynamics in the dendritic shaft as it does in spines, we imaged the polymerizing ends (+ ends) of MTs in neurons expressing EB3-EGFP. Whereas spines were invaded more frequently by + ends following c-LTP induction, + end dynamics in the shaft were unchanged. Conversely, incubation with the calcium chelator BAPTA-AM resulted in fewer + ends entering spines but had minimal effects on + end dynamics in the shaft. Although + ends often polymerized long distances in the shaft (~10μm), those that invaded spines usually traversed much shorter distances before entering spines. These results suggest that MT-spine invasions are primarily instigated by + ends already in close proximity to stimulated spines, with the larger MT population unaffected by synaptic activity or internal calcium.

It remains unclear how NMDAR activation promotes MT entry into spines. Post-synaptic signals may directly influence + end dynamics near spines, *e.g.* promoting MT rescue or inhibiting catastrophe. Alternatively, activity may trigger structural changes in spines that promote MT entry. Since LTP enhances filamentous actin (f-actin) levels in spines, we speculated that stabilization of the spine actin network might promote MT entry. To test this, we imaged cells before and after treatment with the f-actin stabilizing drug jasplakinolide. MT-spine invasions occurred more frequently in the presence of jasplakinolide, suggesting that stabilization of f-actin is sufficient to induce MT entry into spines. This finding supports a “synaptic capture” model, in which activity-dependent stabilization of actin filaments promotes their association with MT + ends in the neighboring shaft, thereby permitting MT polymerization into spines.

ON-CHIP MICRO-ENGINEERED TOPOLOGIES INFLUENCE NEURITE OUTGROWTH IN VITRO

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Multi electrode arrays have been used since the seventies for the study of electrophysiological properties of neuronal cells and their connectivity in a neuronal network. This technology avoids invasiveness encountered by patch clamp methods, and allows for high throughput measurements. However, the signal-to-noise ratio (SNR) and the lack of control on the neuronal connectivity are thus far still inadequate.
Imec works towards a subcellular-sized, three-dimensional micronail electrode array platform to achieve a better contact between the neuronal membrane and the electrodes in order to increase the SNR and to be able to interface on the subcellular scale. Underneath the surface topology of the electrodes an active chip is present to measure and process neuronal signals. With these new technologies it should become possible to study synaptic strengths and neuronal connectivity on the single-cell level, a much-desired feature in present neuronal research.

One of the challenges in the development of the chips is to design the micronails in such a way that neurite outgrowth and connectivity can be accurately controlled. Therefore, we performed a morphological analysis of E17 mouse hippocampal neurons on 3 µm-high micronail arrays. Observations indicate that neuronal morphology alters when growing on nail substrates: intracellular cytoskeleton proteins such as actin filaments and microtubules densify around the nail shaft, membrane staining shows this close contact as well. Initial speed of neurite outgrowth (4 to 30h after plating) was investigated on micronail arrays with different parameters (diameter and spacing of the nails). On the surfaces that appear to provide maximal guidance, total neurite outgrowth is 1.5 times faster in the first 30h compared to a flat surface. Also, the angles of neurite growth patterns relative to the substrate were quantitatively examined. Because of the hexagonal shape of the nails, neurite outgrowth is maximally confined at an angle of 60°, whereas neurites on flat surfaces show random spreading.

These results show that our surfaces have a clear impact on the direction of neuronal growth and behavior. In the future, these engineered substrates could be used for axon guidance and regeneration studies, and implemented in a platform for the study of neuronal connectivity.

INVOLVEMENT OF DENDRITE ARBORIZATION AND SYNAPSE MATURATION (DASM)-1 IN INHIBITORY SYNAPSE DEVELOPMENT

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The immunoglobulin superfamily member Dendrite arborization and synapse maturation-1 (Dasm1) was previously shown to be required for dendritic differentiation of cultured hippocampal neurons and for excitatory synapse maturation in hippocampal organotypic slice cultures. To investigate the in vivo functions of Dasm1 in the developing brain, we generated mutant mice carrying a null allele of the Dasm1 gene. We find that lack of Dasm1 does not affect hippocampal dendrite growth and branching in vitro and in vivo. Instead, lack of Dasm1 specifically interferes with inhibitory, but not excitatory synapse development.
Single cell RT-PCR analysis revealed that Dasm1 is expressed not only by principal neurons but also by inhibitory interneurons in the hippocampus. We therefore recorded mIPSCs in CA1 pyramidal cells where frequencies but not amplitudes of mIPSCs as well as sIPSCs of P15-20 mice were significantly reduced. In P5-6 mice, mIPSC frequencies were reduced to an even greater extent. These physiological abnormalities correlated with reduced number of gephyrin-positive puncta in cultured hippocampal neurons from Dasm1$^{-/-}$ mice implicating a reduction in inhibitory synapses. Dasm1$^{-/-}$ mice exhibited reduced activity in novel object exploratory behavior which would be consistent with decreased inhibition and increased anxiety in these mice. In contrast to the defects in inhibitory synapses, the density of excitatory synapses in the CA1 region was normal in Dasm1$^{-/-}$ mice. Extracellular recordings in hippocampal slices from Dasm1$^{-/-}$ and control mice did not show any differences in input-output-fields, E/S-coupling, paired pulse stimulation and long term potentiation. In whole cell recordings, neither frequencies nor amplitudes of mEPSCs of both P15-20 and P7-8 mice were altered. These findings indicate that Dasm1 is dispensable for excitatory synapse development in vivo. To address the mechanism by which Dasm1 might regulate inhibitory synapse development, we generated mice that express an isoform of Dasm1 lacking the intracellular domain. These Dasm1$^{\Delta C/\Delta C}$ mice show normal mIPSCs and sIPSCs indicating that the extracellular domain of Dasm1 is sufficient for its action. Using a cell-aggregation assay, we show that full-length Dasm1 and Dasm1ΔC mediate homotypic binding and cell aggregation, suggesting that Dasm1 acts as a cell adhesion molecule (CAM). These results suggest that Dasm1 is involved in establishing/maintaining inhibitory contacts in the hippocampus possibly by its CAM-like activity.

MOLECULAR SPECIFICATION OF THALAMOCORTICAL CONNECTIVITY

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The highly specific pattern of connectivity between thalamic nuclei and cortical areas is established through sucessive steps, including: guidance through intermediate areas, topographic sorting of axons, selection of specific cortical areas, interactions with subplate cells during a "waiting period", invasion of the developing cortical plate and selection of specific target cells in appropriate layers.

We have been investigating the functions of Class 6 transmembrane Semaphorins and the interacting Plexin-A proteins in this system. Sema6A is required to specify the guidance of axons from the dorsal lateral geniculate nucles (dLGN), at an intermediate choice point in the internal capsule. Sema6A-positive thalamic axons respond positively to PlxnA2 and PlxnA4, which are expressed along the presumptive route of the thalamic axons in the internal capsule. At the same time, Sema6A acts as a repulsive cue to restrict axons from entering the ventral telencephalon.

In Sema6A mutants, or PlxnA2;PlxnA4 double mutants, dLGN axons project into the ventral telencephalon instead of turning dorsally to enter the cortex. As a consequence, somatosensory (VB) thalamic axons invade presumptive visual cortex at early stages. At later stages, however, many dLGN axons find their way to visual cortex and establish a relatively normal pattern of connectivity. This demonstrates the existence of cues within the cortex itself which specify this connectivity.

We have been searching for such molecules using bioinformatics and expression screening. We have identified several families of transmembrane proteins expressed in specific thalamic nuclei and/or cortical areas and layers, including the Odz, Neto, Kirrel, Elfn, Lrtm, Calsyntenin, Igsf9, Sidekick and several novel gene families. Functional analyses in knockout mice demonstrate a contribution of Neto 1 and 2, which are expressed in complementary sets of thalamic nuclei, in the specific connectivity of these nuclei with their target areas.

In addition, we have found that Sema6A, Sema6B, PlxnA2 and PlxnA4 all play later roles in the invasion of thalamic axons into the cortical plate, in the selection of appropiate target layers and in the segregation of thalamic axon terminals. Through these approaches we are beginning to unravel the cellular processes and molecular logic specifying thalamocortical connectivity.

CANONICAL NF-κB SIGNALING CONTROLS THE EARLY NEURAL ASYMMETRIC DIVISION OF NEURAL STEM CELLS

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Neural stem cells (NSCs) are capable of differentiating into committed daughter cells through asymmetric division. However, the detailed mechanisms to initiate the differentiation of NSC and to modulate early neural asymmetric division remain unknown. Here we show that NF-κB signaling initiates the differentiation of NSCs and modulates the early neural asymmetric division. Selective inhibition of NF-κB signaling blocked the NSC differentiation and the early neural asymmetric division, and maintained undifferentiated NSCs. The induction of asymmetric division by NF-κB signaling occurred through the inhibition of C/EBPβ expression, ahead of β-catenin-modulated asymmetric division during neurogenesis. Our data reveal a novel function of NF-κB in modulating neural differentiation and suggest that pathologic activities of NFκB are able to disturb neurogenesis.

MECHANOTRANSDUCTION DURING AXON CHEMOATTRACTION TO NETRIN-1

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Axons are guided to their target by proteins distributed along their trajectory. These proteins can be transmembrane (e.g. ephrin-Bs and sema-1, -4, -5 & -6), chemically linked to the membrane (e.g. ephrin-As and sema-7) or secreted into the extracellular space (e.g. netrins, BMPs, sema-3 and slits). Although secreted, the netrin-1 cue becomes attached to the extracellular space and provides traction for extending axons (1). In addition to providing traction, this mechanical interaction may initiate intracellular signalling cascades required for chemoattraction to netrin-1. For instance, phosphorylation of the substrate domain of p130CAS occurs upon mechanical tension and is known to be required for chemoattraction to netrin-1 (2,3). Similarly, focal adhesion kinase (FAK) is required for chemoattraction to netrin-1 and there is evidence that it is activated by mechanical force (4-7). Here we explore whether p130Cas and FAK function as mechanotransducers during chemoattraction to netrin-1.

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INTEGRATION OF INTEGRIN AND TRK RECEPTOR SIGNALS BY DUAL FAK-SRC ACTIVITIES IN GROWTH CONES.

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The ability of extending axons to navigate using combinations of extracellular cues is essential for proper neural network formation. One intracellular signaling molecule that integrates convergent signals from both extracellular matrix (ECM) proteins and growth factors is focal adhesion kinase (FAK). Analysis of FAK function shows that it influences a variety of cellular activities including cell motility, proliferation and differentiation. Recent work in developing neurons has shown that FAK and Src function downstream of both attractive and repulsive growth factors, but little is known about the effectors or cellular mechanisms which FAK controls in growth cones on ECM proteins. We report that FAK functions downstream of Brain Derived Neurotrophic Factor (BDNF) and laminin in the modulation of point contact dynamics, phosphotyrosine (PY) signaling at filopodial tips, and lamellipodial protrusion. Stimulation of Xenopus spinal neurons on laminin with BDNF leads to a rapid and sustained increase in PY at filopodial tips, as well as an increase in the dynamics of lamellipodial protrusions. Additionally, we show that BDNF stimulation accelerates paxillin-containing point contact turnover and formation. Knockdown of FAK function either with a FAK anti-sense morpholino or by expression of FRNK, a dominant-negative FAK isoform, blocks all aspects of the response to BDNF, including the acceleration of point contact dynamics. On the other hand, expression of specific FAK point-mutants selectively disrupts certain aspects of the response to BDNF, underlining the pivotal and diverse role of FAK as a signal integrator. Preliminary studies also show that the repulsive response to Slit2 involves the inactivation of FAK and Src and the acute disassembly of paxillin-containing point contacts. By using FAK mutants which fail to convey certain signals, we can determine the relative contribution of different cellular mechanisms to growth cone turning *in vitro* and axon guidance *in vivo*. Differential regulation of these mechanisms within growth cones downstream of distinct axon guidance cues are likely mechanisms that allow growing axons to navigate the complex environment of a developing embryo.

DROSOPHILA FOXO NEGATIVELY REGULATES MICROTUBULE STABILITY AND IS REQUIRED FOR PROPER NEUROMUSCULAR JUNCTION MORPHOLOGY AND FUNCTION.

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Drosophila FoxO encodes a conserved transcription factor implicated in many important cellular functions including metabolism, apoptosis, differentiation, neuronal excitability, and autophagy. The roles of FoxO in oxidative stress response and longevity, however, have been most extensively studied. Activated FoxO is known to promote oxidative stress resistance across species. Additionally, life span extension due to mutation in insulin receptor is completely dependent on intact FoxO activity, and tissue-specific FoxO overexpression in flies is sufficient to promote longevity. Thus far, FoxO function in the above processes have been extensively studied at the organismal level, yet the cellular mechanism(s) responsible for its diverse actions remain(s) elusive. To investigate a role for *foxO* in *Drosophila* neuronal development, we first characterized FoxO expression. Unexpectedly, we find the FoxO expression profile to be largely specific to motorneurons (MNs), as FoxO and the MN marker pMad exhibit extensive co-localization. To determine whether *foxO* is required for MN development, we assayed MN differentiation in FoxO loss-of-function (LOF) and gain-of-function (GOF) mutants. FoxO LOF larvae exhibit aberrant NMJ morphology characterized by dramatically increased microtubule (MT) looping and a smaller number of enlarged synaptic boutons. The former phenotype has been reported to associate with hyperstable and hypostable MT cytoskeleton. To clarify the directionality of the MT phenotype in FoxO LOF mutants, we treated wild-type larvae with a MT-stabilizing drug taxol, which phenocopied FoxO LOF MT looping phenotype. Consistent with the possibility that FoxO negatively regulates MT stability, we detect increased levels of acetylated α-tubulin, a stable-MT marker, at the FoxO LOF NMJ. The finding that FoxO is involved in regulating MT stability led us to hypothesize that the morphological defects at the *foxO* mutant NMJ may be due to hyperstable MTs. Consistently, we can rescue morphological defects seen in FoxO LOF larvae by removing one copy of MAP1B. Contrary to the FoxO LOF phenotype, MN-specific overexpression of *foxO* leads to reduced MT stability, increased number of synaptic boutons, and decreased bouton size. These structural defects led us to ask whether foxO is also required for proper NMJ function. Consistently, we observe a reduction in evoked neurotransmitter release and a defect in FM 1-43 dye uptake in FoxO LOF larvae. We will present our ongoing efforts to characterize foxO targets that mediate its effect on MT stability at the NMJ.

AXON ARBORIZATION AND SYNAPTOGENESIS IN A SEROTONERGIC NEURON IN *C. ELEGANS*.

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While serotonergic neurons constitute only 1 in 1 million of all CNS neurons, they form 1 in 500 cortical axon terminals in the rat[1]. This dense innervation of the cortex is achieved through the development of elaborate serotonergic axon arbors which contain neurosecretory synapses[2]. This process allows serotonin neurons to innervate their targets and effect their neuromodulatary role in the nervous system. How this developmental process ensues, and how it is regulated, is not understood.

We have established a system in *C. elegans* to understand this question. The serotonin biosynthesis pathways are well conserved in *C. elegans*, and we can visualize the neurodevelopment of serotonergic neurons in vivo, in real time and with single cell resolution. We have focused our studies on the main serotonergic neuron in the nematode, NSM. Like vertebrate serotonin neurons, NSM forms elaborate axon arbors that contain extrasynaptic serotonin release sites. These arbors develop in a precise region of the animal's pharynx, and at a specific time during the animal's development.

We have used molecular genetic and cell biological approaches to identify the molecular signals that control this development. We have found that it is controlled by UNC-6/Netrin, the Netrin receptor UNC-40/DCC, and the repulsive Netrin co-receptor UNC-5. We have also shown that intermediate signaling molecules UNC-34/Ena/VASP and MIG-10/Lamellipodin are required for axon arbors in NSM.

Mig-10 can be alternatively spliced into 3 different isoforms: MIG-10A, MIG-10B, and MIG-10C. While these isoforms differ only by short unique exons at the N-terminus, they have distinct functions in directing neurodevelopmental processes. Here we show that the MIG-10A and MIG-10C isoforms act cell-autonomously in NSM to promote axonal arborization. This is a novel role for these isoforms; and these data contrast with findings that MIG-10B functions cell-autonomously to mediate presynaptic assembly.[3]

With these molecular handles, we are now exploring how these molecules are temporally and spatially controlled to instruct axon arborization and synaptic assembly in NSM. Specifically, we are using real time microscopy to visualize, in live animals, the dynamic localization of these molecules with respect to axon arborization and synaptogenesis.

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CLIPS REGULATE NEURONAL POLARIZATION THROUGH MICROTUBULE AND GROWTH CONE DYNAMICS

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Axon formation is a hallmark of initial neuronal polarization. This process is thought to be regulated by enhanced microtubule stability in the subsequent axon and changes in actin dynamics in the future axonal growth cone. Here, we show that the microtubule end-binding proteins Cytoplasmic Linker Protein (CLIP) 115 and CLIP 170 were enriched in the axon and extended into the actin rich domain of the growth cone. CLIPs were necessary for axon formation and sufficient to induce an axon. The regulation of axonal microtubule stabilization by CLIPs enabled the protrusion of microtubules into the leading edge of the axonal growth cone. Moreover, CLIPs positively regulated growth cone dynamics and restrained actin arc formation, which was necessary for axon growth. Thus, our data suggest that CLIPs govern neuronal polarization by controlling the stabilization of microtubules and growth cone dynamics.

TSC-MTORC SIGNALING MEDIATES EPHA-INDUCED INHIBITION OF PROTEIN TRANSLATION AND AXON GUIDANCE

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Accumulating evidence indicates a role for local protein synthesis in growth and navigation of developing axons. However, the signaling cascades that link extracellular axon guidance to the protein translational machinery are unclear. We have been studying a human disease, Tuberous Sclerosis Complex (TSC), to probe the neuronal signaling mechanisms controlling the protein synthesis machinery. TSC is caused by mutations in the TSC1 or TSC2 genes, which encode a protein complex that regulates protein synthesis by inhibiting mTORC1 activity. We hypothesized that disruption of the protein translation machinery in TSC1/2 deficient neurons and axons contributes to the pathogenesis of neurological symptoms such as epilepsy, mental retardation and autism in TSC patients. Recently, we showed that Tsc/mTORC pathway components are expressed in CNS axons and homozygous Tsc1 inactivation results in the formation of multiple axons during early neuronal development (Choi et al., 2008). Furthermore, diffusion tensor imaging (DTI) in TSC patients demonstrates axonal disorganization in several pathways including the visual system (Krishnan et al., 2010). Here, we report that in mice Tsc2 haploinsufficiency causes aberrant retinogeniculate projections due to defects in EphA receptor-dependent axon guidance. We also find that EphA receptor activation by ephrin-A ligands in neurons leads to inhibition of ERK1/2 kinase activity and thus decreased inhibition of Tsc2 by ERK1/2. Thus, ephrin stimulation inactivates the mTOR pathway by enhancing Tsc2 activity. As a consequence of ERK and mTOR inactivation, ephrin-A inhibits *de novo* protein translation in acutely isolated neurites, suggesting that local Eph-signaling regulates local protein translation in dendrites and axons. Interestingly, both mTORC1 inhibition and ephrin-A stimulation partially inhibit local protein synthesis to a similar extent. Constitutive mTORC1 activation in Tsc2 deficient neurons reduces ephrin-induced growth cone collapse. Thus, our results demonstrate that the EphA receptors modulate local protein synthesis and disruption in TSC-mTORC pathway causes abnormal axon pathfinding in $Tsc2^{+/-}$ mouse model. Using multiple approaches, we are now pursuing the identity of specific mRNAs whose translation is regulated by EphA receptors and the mTOR pathway in axons.

SYNAPSE FORMATION OF OSN AXONS WITH M/T CELL DENDRITES IN MUTANT MICE THAT HAVE DEFECTS IN GLOMERULAR MAP FORMATION

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Odor signals received by odorant receptors in the olfactory epithelium (OE) are represented as an odor map of activated glomeruli in the olfactory bulb (OB), and conveyed to the olfactory cortex via second-order neurons, mitral/tufted (M/T) cells. In the mouse olfactory system, much of axon pathfinding and sorting appears to occur autonomously by axon-axon interactions of olfactory sensory neurons (OSNs). However, the glomerular map still requires correct orientation on the OB and right connections to M/T cells to generate a functional map.

As for the dorsal (D)-domain of the OB, there seems to be multiple modalities for distinct innate responses, such as fearful, aversive, attractive and social behaviors, which are mediated by genetically-programmed, hard-wired circuits. Also, in the ventral (V)-zone of the OE, several subsets of OSNs have been reported, which respond to specific ligands such as CO_2, that induce specific innate behaviors. Therefore, proper synapse formation of OSN axons with M/T cell dendrites is quite important for the appropriate conversion of sensory stimuli to functional and behavioral responses that are mediated by higher cortical neurons.

Are M/T cells naïve with respect to projections that drive innate behaviors? What mediates the synapse formation between the OSN axons and primary dendrites of M/T cells? Are M/T cells instructed by OSNs? What are the post-synaptic events involved in these processes? To address these questions, we have analyzed synapse formation of M/T cell dendrites in various mutant mice that have defects in glomerular map formation. For example, in the ΔD mutant mice, the D domain of the OB remained devoid of glomerular structures with persistence of M/T cells in the vacant areas in the OB. This finding is unexpected, because in other sensory systems, such as retinotectal projection, competing axons eventually occupy vacant projection sites. In the olfactory system, the OB may not simply be a projection screen to form a glomerular map, but may, instead, have region-specific functions that are genetically predetermined by the second-order neurons.

We also performed chemical ablation of OSN axons. Intraperitoneal injection of dichlobenil removed OSNs from the D region of the OE. We have studied the synapse formation of regenerating OSN axons with pre-existing primary dendrites of second-order neurons.

NEUROTACTIN AND *ABELSON* INTERACT WITH THE NETRIN-FRAZZLED PATHWAY TO PROMOTE MIDLINE AXON CROSSING IN *DROSOPHILA*

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Netrin and DCC/Frazzled(Fra) constitute a conserved ligand-receptor pair that can induce outgrowth and attractive axon turning. To gain insight into the mechanism of Frazzled-mediated attractive axon guidance, we have conducted a sensitized genetic screen for novel mediators of axon guidance at the *Drosophila* midline. Expression of a dominant-negative Fra receptor causes dose-dependent defects in axon attraction that are sensitive to levels of the endogenous *fra* gene as well as, presumably, other genes in this pathway. We have uncovered a genomic interval whose heterozygous deletion strongly enhances defects seen in this sensitized genetic background. This enhancement is due to loss of function of two genes. One of these genes encodes the Abelson tyrosine kinase (*abl*), while the other, *neurotactin* (*nrt*), encodes a cholinesterase-like heterophilic adhesion molecule. Others have shown that Nrt collaborates with other cell adhesion molecules to ensure proper midline axon guidance. We find that *nrt* loss-of-function alleles strongly enhance *fra* hypomorphic combinations, suggesting that Nrt contributes to midline attraction. *Nrt* also appears in part to act independently of the *fra* pathway suggesting a role for cholinesterase-like adhesion molecules in multiple axon guidance pathways.

To understand the role of *abl* in midline attraction we have undertaken a combination of molecular and genetic approaches. *Abl* loss-of-function can dominantly enhance commissural guidance defects in *fra* hypomorphic mutants. To more broadly understand the role of tyrosine kinase signaling in Netrin-mediated axon attraction, we have also examined the role of the two *Drosophila src* genes, *src42a* and *src64b*. In vertebrates, Src family kinases transduce Netrin signals by phosphorylating a key tyrosine residue on the DCC receptor, likely promoting recruitment of downstream effector(s). We have tested this model to determine the mechanism of Netrin signal transduction in *Drosophila*. Expression of rat DCC fully rescues *fra* loss of function in commissural neurons, but this key tyrosine residue is not required for rescue. Thus, this tyrosine phosphorylation event is not required in *Drosophila* for commissural axon attraction. Additionally, genetic interactions suggest that *src* family kinases inhibit midline axon crossing, in contrast to their role in vertebrates, while *abl* promotes crossing. Based on these results, we suggest a distinct mechanism of Netrin-mediated signal transduction, possibly through the regulation of downstream effectors via tyrosine phosphorylation.

TSUKUSHI IS A NOVEL WNT INHIBITOR INVOLVED IN THE REGULATION OF NEURONAL STEM CELLS AND THE ANTERIOR COMMISSURE FORMATION

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Wnt signalling orchestrates multiple aspects of central nervous system development, including cell proliferation and cell fate choices. In chick retina, Wnt2b is expressed in both the surface ectoderm and the retinal pigmented epithelium at optic cup stage. At later stage, Wnt2b is expressed in the marginal-most tip of the embryonic chick retina. Using a clonal assay in retinal re-aggregation cultures, along with overexpression studies, it was proposed that Wnt2b plays a role in the proliferation of retinal progenitor cells without cell differentiation (Kubo et al., 2005).
We have identified a BMP antagonist, Tsukushi (TSK), which is a soluble molecule containing 12 leucine-rich repeats and belongs to the Small Leucine-Rich Proteoglycan family (Ohta et al., 2004). TSK is expressed in the primitive streak and Hensen's node during chick gastrulation and involved in their formation (Ohta et al., 2004; Ohta et al., 2006). TSK is also involved in the neural crest specification of Xenopus embryo by regulating BMP and Delta-1 activities at the boundary between the neural and the non-neural ectoderm (Kuriyama et al., 2006). Further, TSK contributes to germ layer formation and patterning in Xenopus development by modulating Xnr2, FGF, and BMP signalling (Morris et al., 2007).
When Wnt2b was over-expressed into chick optic vesicle at stage 10, Wnt2b induced prolonged proliferation of retinal progenitor cells in vitro. However, when Wnt2b was co-over-expressed with TSK, which is also expressed in the marginal-most tip of the chick retina, the proliferative activity of Wnt2b was almost inhibited. Biochemical analyses showed that TSK functions as a Wnt signaling inhibitor by direct binding to Frizzled4 at the extracellular region. In the mouse retina, TSK is expressed in the ciliary body (CB) in which retinal stem/progenitor cells are located, and targeted disruption of TSK in mouse resulted in the expansion of the CB and an increase in the number of retinal spheres. Using gain- and loss-of function studies, we uncover a new crucial role for TSK in maintaining the growth and undifferentiated properties of retinal stem/progenitor cells as a niche molecule. We also found that TSK is also involved in the anterior commissure formation. I would like to discuss about this finding.

ODORANT RECEPTOR-DERIVED NEURONAL FIRING AND BASAL ACTIVITIES OF OLFACTORY SENSORY NEURONS DIFFERENTIALLY REGULATE OLFACTORY MAP FORMATION

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In the mouse olfactory system, olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) converge their axons into a specific set of glomeruli in the olfactory bulb (OB). This olfactory map is generated by two successive processes, global targeting and local sorting of OSN axons. A remarkable feature of axonal projection in the mammalian olfactory system is that ORs play important roles in both processes by regulating transcriptional levels of two distinct types of axon guidance/sorting molecules. Axon guidance molecules, such as Neuropilin-1(Nrp1), determine global positioning of glomeruli along the anterior-posterior (A-P) axis. In contrast, homophilic adhesion molecules, such as Kirrel2, regulate local sorting of like axons to form a distinct glomerular structure. However, it remains unclear how ORs can regulate the expression of these two distinct types of axon guidance/sorting molecules. It has been well established that neuronal firing has an instructive role in the topographic map formation in various parts of the brain. To examine whether the neuronal firing in OSNs is required for the glomerular map formation, we utilized the inward rectifying potassium channel, Kir 2.1. Overexpression of Kir2.1 hyperpolarizes neurons and thus, inhibits the firing of action potentials. In the mouse in which Kir2.1 is overexpressed specifically in OSNs, OSN axons fail to form distinct glomerular structures. In the Kir2.1 overexpression mouse, *Kirrel2* is downregulated, but *Nrp1* is not, suggesting that OR-derived neuronal firing contributes to the local sorting of OSN axons. Many G protein coupled receptors (GPCRs) are known to generate the so-called basal activity in the absence of agonists. We assume that the basal activity of ORs also participate in the glomerular map formation. Due to the absence of detailed functional analyses of ORs *in vitro*, it has been difficult to study a possible role of OR-derived basal activities in the glomerular map formation. Key residues in beta-2 adrenergic receptor (β2AR) have been identified for the basal activity, which shares many functional similarities with OR molecules. OSNs expressing β2AR in place of ORs can form a specific glomerulus at a unique location in the OB. Taking advantage of this β2AR-instructed glomerulus formation, we have analyzed axonal projection of OSNs that express the mutant type β2AR with the altered levels of basal activity. Glomerular locations changed accordingly along the A-P axis in the OB. The basal activity of β2AR affected expression levels of Nrp1, but not that of Kirrel2. These results indicate that two distinct OR-derived signals, one which is the basal activity and the other which is neuronal firing, separately regulate the two distinct processes of glomerular map formation, i.e., global targeting and local sorting of OSN axons, respectively.

THE CYTOSKELETAL REGULATOR GENGHIS KHAN IS REQUIRED FOR COLUMN-SPECIFIC BUT NOT LAYER-SPECIFIC TARGETING IN THE *DROSOPHILA* VISUAL SYSTEM.

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Different neuronal cell types can often be distinguished by their specific patterns of synaptic connections. While many molecules have been identified that are important for synaptic specificity, it is still not well understood how closely related cell types choose distinct synaptic targets. Here we describe a novel cytoskeletal regulator that is critical for a specific type of axonal projection, in a single cell type.

In the visual system, axonal and dendritic projections are broadly organized into columns and layers, where columns are arranged in a retinotopic map, and different layers within each column process different types of visual information. In *Drosophila*, photoreceptor axons display both column and layer specificity. One subset of photoreceptors, the R1-R6 cells, innervate specific columns within one ganglion, the lamina, while two other cells, designated R7 and R8, target distinct layers within a second ganglion, the medulla. Many molecules are known to control these targeting choices, but most are required in both subsets of photoreceptors. Here we identify the first factor, the kinase Genghis khan (Gek), that is required only for columnar but not layer specific targeting.

Gek was identified in a forward genetic screen for genes required for visual system behavior and connectivity. Gek is expressed in all photoreceptor axons at developmentally appropriate times, and somatic mosaic studies demonstrate that Gek is required cell-autonomously in R1-R6 cells for these axons to choose their correct target columns in the lamina. In addition, structure-function studies demonstrate that Gek is normally regulated by auto-inhibition of its kinase activity, and that this regulation normally serves to restrict Gek activity to the axon. Intriguingly, however, while Gek has no normal role in R7 or R8 target selection, over-expression of Gek in R7 neurons can alter their targeting choices, causing them to innervate inappropriate layers. We propose that while column and layer-specific targeting mechanisms use many of the same effectors, columnar targeting utilizes Gek as a specialized modulator of cytoskeletal organization.

SEMA6A REPULSIVE SIGNALING THROUGH ITS PLEXINA4 RECEPTOR CONTROLS LAMINA-SPECIFIC NEURONAL CONNECTIVITY IN THE VERTEBRATE RETINA

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In the vertebrate retina, establishment of precise synaptic connections among different retinal neuron cell types is essential for processing visual information and for accurate visual perception. Three distinct neuronal cell types, retinal ganglion cells, amacrine cells, and bipolar cells, form synapses in the inner plexiform layer (IPL). Each neuronal cell type includes multiple subclasses, and each subclass has a characteristic laminar connection pattern within the IPL (conventionally divided into 5–10 parallel sublaminae). However, the molecular mechanisms governing distinct retinal subtype targeting to specific sublaminae within the IPL remain to be elucidated. Here, we show that the transmembrane semaphorin Sema6A signals through its receptor PlexinA4 to control lamina-specific neuronal connectivity in the mouse retina. Mice with null mutations in Sema6A or PlexinA4 exhibit defects in stereotypic lamina-specific neurite arborization of dopaminergic tyrosine hydroxylase (TH)-expressing amacrine cells, and also calbindin-positive cells in the IPL. In addition, Sema6A and PlexinA4 genetically interact in vivo with respect to the regulation of dopaminergic amacrine cell laminar targeting. Moreover, we find that intrinsically photosensitive retinal ganglion cells (ipRGCs) of the M1 type, known synaptic partners of dopaminergic amacrine cells, also exhibit aberrant dendritic stratification in the IPL in Sema6A and PlexinA deficient mice, misprojecting to the same regions as we observe for mutant dopaminergic amacrine cells in these mutants. Expression analyses demonstrate that Sema6A and PlexinA4 protein are expressed in a complementary fashion throughout postnatal retinal development. Specifically, Sema6A is strongly expressed in ON sublaminae of the IPL, whereas PlexinA4 protein is expressed in retinal cell types that stratify in OFF sublaminae of the IPL. These findings suggest a mechanism by which initial neuronal targeting to larger subdivisions of the IPL in the vertebrate retina is directed by repulsive transmembrane guidance cues present on neuronal processes, allowing for subsequent adhesive interactions that refine targeting to individual IPL sublaminae.

ACTIVITY-DEPENDENT DEVELOPMENT OF INHIBITORY AXON TERMINALS: ROLE OF PRESYNAPTIC $GABA_B$ RECEPTORS AND NEUREXIN ISOFORMS

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The development of inhibitory synapses and innervation patterns in neocortex is regulated by neural activity, but the underlying cellular mechanisms are poorly understood. We previously showed that GABA signaling acts beyond synaptic transmission and regulates inhibitory synapse development. Thus similar to glutamate signaling at developing excitatory synapses, GABA may coordinate pre- and post-synaptic maturation at inhibitory synapses. Here we address the role of presynaptic $GABA_B$ receptors ($GABA_BR$) and neurexin isoforms.

By pharmacologically blocking $GABA_B$Rs *in vitro* and genetic deletion of $GABA_B$R in parvalbumin-containing (Pv) basket interneurons in vivo, we found that presynaptic $GABA_B$Rs cell-autonomously regulate axon branching and density of presynaptic boutons. To examine the underlying cellular mechanism, we imaged the synaptic dynamics in Pv basket interneurons in live cortical organotypic cultures by 2-photon microscopy. We found that small immature presynaptic boutons were unstable, appear and disappear repeatedly at predefined sites along axons. Reduced GABA signaling in Pv cells by GAD67 knockout let to an increase in the fraction of small unstable boutons, while GAD67 overexpression stabilized unstable small boutons. Furthermore, $GABA_B$R agonists rescued the unstable boutons in GAD67 KO cells, while $GABA_B$R antagonists and single PV+ cell deletion of $GABA_B$R decreased bouton stability.
These results suggest that presynaptic $GABA_B$Rs act as a local sensor for the strength of GABA release in regulating activity dependent inhibitory synapse maturation and axon morphogenesis.

We further examined whether and how GABA signaling regulates a key synaptic adhesion system, the neurexins. We developed a method to study the sub-axonal localization, dynamics, and regulation of neurexin isoform NRX1α and NRX1β in developing Pv+ axons and synapses. Both isoforms are delivered to presynaptic terminals but show significant and different turnover rate at the membrane. While NRX1α is highly diffuse along developing axons and filopodia, NRX1β is strictly anchored at terminals through binding to postsynaptic ligands. The turnover rate of NRX1β is attenuated by neural activity and presynaptic GABAB receptors. Neurexins thus are intrinsically dynamic, but is stabilized by local transmitter release. Such an activity-adjusted adhesion system seems ideally suited to rapidly explore and validate synaptic partners guided by synaptic transmission. Together, these results reveal novel functions of presynaptic $GABA_B$R signaling in regulating synaptic adhesion system and in activity-dependent development of inhibitory synapses.

CONTEXT SPECIFIC MECHANISMS IN LAR AND LIPRIN DEPENDENT SYNAPTOGENESIS

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Synapse formation is triggered by interactions between adhesion molecules on the axon and its postsynaptic target, and involves the assembly of a complex network of cytoskeletal and adaptor molecules in the presynaptic active zone. Central to this network is the Liprin-α family of scaffolding proteins, which were first isolated as binding partners of the receptor protein tyrosine phosphatase LAR. These Liprins act as adaptor proteins involved in the regulation of neurotransmitter release and axonal transport. Since *Lar* and *Liprin-α* mutants have similar neuronal phenotypes, a complex containing both proteins may regulate synaptogenesis. In *Drosophila*, LAR and Liprin-α are required for the color-sensitive photoreceptors R7 and R8 to project to distinct layers of the medulla; in the absence of either protein, R7 prematurely terminates at the R8 target layer. At the neuromuscular junction (NMJ), *Liprin-α* and *Lar* mutants show reduced numbers of synaptic boutons, enlarged active zones, and impaired transmission.

We found that LAR signals via different mechanisms in R7 photoreceptors and in motor neurons. NMJ synaptic growth requires catalytic LAR phosphatase activity which is dispensable for R7 targeting. This process instead relies on LAR dimerization. Based on extracellular domain deletion studies, we deduced that LAR activity in R7 is controlled by a ligand different from the HSPG ligands required for NMJ synaptogenesis. This novel ligand may induce dimerization-based conformational changes in the LAR intracellular domain,thereby altering the availability of protein binding sites and thus controlling the assembly of Liprin-α and other active zone proteins.

We found that Liprin-α can bind to the two other members of the Liprin family, Liprin-β and Liprin-γ. Like *Liprin-α*, *Liprin-β* is required for NMJ growth, and contributes to normal R7 synaptogenesis, although *Liprin-β* mutant R7 axons overshoot their normal target layer rather than retracting to the R8 layer. Genetic interactions demonstrate that both Liprin-α and Liprin-β act through the exchange factor Trio to promote stable R7 target selection. *Liprin-γ* counteracts the functions of the other two *Liprins*; loss of *Liprin-γ* improves the R7 phenotype of *Liprin-α* mutants, and the NMJ phenotype of *Liprin-β* mutants. We are currently investigating whether these antagonistic effects are due to competitive binding. Context-specific LAR signaling and interactions between the three Liprins suggest that motor neuron and photoreceptor synapses differ in their active zone assembly mechanisms.

REGULATORS OF SYNAPTIC REMODELING IN *C. ELEGANS* ARE REVEALED BY IDENTIFICATION OF UNC-55 TRANSCRIPTIONAL TARGETS

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Individual neurons define functional circuits by adopting polarized morphologies with separate axonal and dendritic compartments. These domains may be reorganized by developmental cues or in response to injury but the mechanisms that regulate this plasticity are poorly understood. One example of this phenomenon occurs in the GABAergic motor circuit of *C. elegans*. Dorsal D (DD) motor neurons initially establish inhibitory neuromuscular junctions (NMJs) with ventral muscle. At the end of the first larval stage, DDs reverse polarity to synapse with dorsal muscle (White et. al. 1978). This DD polarity switch is coincident with the birth of VD motor neurons, which make NMJs with ventral muscles. UNC-55 is a COUP family nuclear hormone receptor that is normally expressed in VD motor neurons (Shan et. al., 2005). In *unc-55* mutants, VD motor neurons are remodeled to mimic the dorsal polarity of DD motor neurons (Walthall and Plunkett, 1995). Thus, UNC-55 appears to function as a transcriptional switch to block the DD remodeling program in VD motor neurons. To identify these synaptic remodeling genes, we compared cell-specific microarray profiles of wild-type vs. *unc-55* GABAergic motor neurons and detected 188 enriched transcripts (2X, 1% FDR) in the *unc-55* data set. RNAi of these candidate UNC-55 targets revealed 21 conserved genes that partially suppress the Unc-55 remodeling defect ($p<0.01$), as visualized by restoration of ventral GABAergic NMJs marked with a GFP-labeled synaptic vesicle protein. Suppressors of the Unc-55 synaptic defect include genes encoding a wide array of protein families (ion channels, cytoskeletal components, cell-cell signaling molecules, transcription factors) and are therefore suggestive of a complex remodeling program. Cell-specific RNAi knockdown in GABA neurons of one of these genes, the Iroquois homeodomain transcription factor, *irx-1*, retards the normal remodeling program in DD motor neurons as well as VD polarity reversal in *unc-55* mutants. These effects are consistent with the idea the IRX-1 activates expression of downstream targets that drive GABAergic motor neuron remodeling. Current experiments are designed to parse the genetic pathway that drives synaptic remodeling by identifying *unc-55*-regulated genes in our data set that act downstream of *irx-1*. Future work will define the cellular roles of these *unc-55* and *irx-1* regulated genes in the mechanism of synaptic remodeling.

OTX2 HOMEOPROTEIN TRANSFER AND SIGNALING IN VISUAL CORTEX PLASTICITY

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Homeoproteins (HP) transcription factors play fundamental roles in development, from embryonic polarity to cell differentiation and migration. Aside from their well established cell autonomous activity, HP also undergo intercellular transfer. In the latter context, we have shown that postnatal Otx2 HP internalization by parvalbumin (PV) GABAergic interneurons initiates their maturation and opens the critical period for ocular dominance in the visual cortex (Sugiyama et al., Cell, 2008). This work has provided the first identified physiological role for HP transfer in vivo.
Accumulation of Otx2 inside PV cells is driven by visual experience. Accordingly, Otx2 can be transported along the visual pathway, from the retina to the visual cortex, supporting the possibility that Otx2 in PV cells takes its origin in the eye. However this eye to cortex transport does not exclude the existence of alternative sources of Otx2. Among them the choroid plexus is an interesting candidate given that it is in the vicinity of the cortex and massively expresses Otx2. To test this idea, we developed a protocol allowing an efficient and specific delivery of active Cre recombinase in choroid plexuses leading to a 50% reduction of the amount of Otx2 protein specifically in the choroid plexuses of Otx2flox/flox mice. Our preliminary analyses indicate that partial Otx2 invalidation in the choroid plexus reduces the levels of PV expression in V1B as well as that of complex sugars that are part of PV cells perineuronal nets (PNNs). This strongly suggests that Otx2 in provenance from the choroid plexus plays a role in PV cell phenotype.
The specific internalization of Otx2 by PV cells and the known affinity of transcription factors for complex sugars led us to study if glycosaminoglycans (GAGs) present in the PNNs play a role in Otx2 capture by PV-cells. This hypothesis is supported by the finding that GAG hydrolysis by Chondroitinase ABC (ChABC) reduces the amounts of endogenous Otx2 into PV cells and reopens ocular dominance plasticity in the adult. We have identified within Otx2 a 15 amino acid GAG-binding domain necessary for Otx2 recognition by PV cells. This domain antagonizes Otx2 binding to target cells in vitro, blocks Otx2 transfer in vivo and reactivates ocular dominance plasticity in the adult, similarly to ChABC treatments.
Taken together, our results suggest that several sources for cortical Otx2 may coexist in the adult and that lowering Otx2 transport into PV cells reopens plasticity in the adult visual cortex. They also demonstrate that the specific addressing of Otx2 to PV cells requires its recognition by complex sugars that are part of the PNNs. It is tempting to speculate that this concept and the possibility to reopen plasticity in the adult is not limited to the visual system.

GENES THAT PROMOTE OR REPRESS AXON REGROWTH AFTER LASER SURGERY IN *C. ELEGANS*

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Understanding the mechanisms of axonal regrowth after injury is of central interest to human health. However the genetic pathways regulating the ability of mature neurons to regrow axonal processes after injury remain little explored. The labor intensive nature of axotomy experiments in vivo has precluded large-scale genetic screens. *C. elegans* neurons display robust regrowth after femtosecond laser axotomy. We have screened several hundred conserved genes for their roles in regrowth of *C. elegans* mechanosensory neurons after axotomy. Each gene is tested using genetic loss or gain of function mutations. We quantitate >10 parameters of axon regrowth 24 h post axotomy. We find approximately 10% of genes screened are required for efficient regrowth. These genes fall into several functional categories, and are notably enriched for genes affecting neuronal excitability, neurotransmission, and synaptic vesicle endocytosis. Most of these genes have no detectable role in developmental axon outgrowth. We also identify over ten genes for which loss of function results in increased regrowth. This regrowth-inhibiting gene set is enriched in extracellular matrix, cell signaling, and cytoskeletal regulators. We will report our analysis of two regrowth-inhibitory pathways involving Slit/Robo signaling and a conserved guanine nucleotide exchange factor (GEF). We show that Slit/Robo signaling impedes regrowth during the phase of axon extension, whereas the GEF affects an earlier stage in growth cone establishment, in part through regulation of microtubules. Previous studies have shown that DLK-1 MAP kinase cascade is essential for regrowth of *C. elegans* axons. We will report our analysis of the interactions between the newly identified pathways and DLK-1 signaling.

PROMOTING AXON GROWTH OVER CNS INHIBITORS BY TARGETING GROWTH CONE CYTOSKELETAL COMPOMENTS

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Mature neurons in the mammalian nervous system have limited capacity to re-establish lost connections through long-distance axon regeneration. Because a major obstacle to axon regeneration is the hostile CNS environment composed of a series of inhibitory molecules, strategies for promoting axon regeneration have largely been aimed at identification and blockade of these inhibitory factors. However, although considerable controversies exist, results from genetic studies suggest that merely counteracting the individual inhibitory components might be insufficient to trigger extensive regeneration. Given that regeneration failure is also attributed to the diminished intrinsic axon growth capacity of mature neurons, alternative methods to promote axon regeneration would be to re-stimulate the growth capacity. Growth cone is the converging target of axon growth regulatory signals and impediments to axon regeneration act primarily on the growth cone to shut down axon assembly. However, little attention has been paid to promoting axon growth by directly targeting growth cone cytoskeleton. In growth cones, retrograde actin flow driven by non-muscle myosin II (NMII) prevents MT protrusion, which is crucial for efficient axon growth. Here, we show that axons from both the CNS and PNS neurons show markedly enhanced growth by manipulation of NMII. Remarkably, inhibition of NMII enables lesioned adult neurons to completely overcome potent CNS inhibitors, such as chondroitin sulfate proteoglycans (CSPGs) and myelin, and robustly grow axons over these inhibitory substrates. We provide evidence that this axon growth promotion occurs via inducing MT protrusion towards growth cone periphery. Furthermore, by using the two-compartment culture platform we demonstrate that local blockade of NMII activity in distal axons is sufficient to allow growth cones to cross the permissive-inhibitory boundary. By directly targeting growth cone cytoskeletal components, our study suggests a possibility of inducing robust axon assembly over potent growth impediments without degrading or blocking the individual components. Thus, developing strategies to directly control the growth cone may provide alternative and efficient methods for promoting axon regeneration.

PTEN DELETION ENHANCES THE REGENERATIVE CAPACITY OF ADULT CORTICOSPINAL NEURONS

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Despite the essential role of the corticospinal tract (CST) in controlling voluntary movements, successful regeneration of large numbers of injured CST axons beyond a spinal cord lesion has never been achieved. Here we demonstrate a critical involvement of PTEN/mTOR in controlling the regenerative capacity of mouse corticospinal neurons. Upon the completion of development, the regrowth potential of CST axons lost and this is accompanied by a down-regulation of mTOR activity in corticospinal neurons. Axonal injury further diminishes neuronal mTOR activity in these neurons. Forced up-regulation of mTOR activity in corticospinal neurons by conditional deletion of PTEN, a negative regulator of mTOR, enhances compensatory sprouting of uninjured CST axons and even more strikingly, enables successful regeneration of a cohort of injured CST axons past a spinal cord lesion. Furthermore, these regenerating CST axons possess the ability to reform synapses in spinal segments distal to the injury. Thus, modulating neuronal intrinsic PTEN/mTOR activity represents a potential therapeutic strategy for promoting axon regeneration and functional repair after adult spinal cord injury.

EPHB SIGNALLING DIRECTS PERIPHERAL NERVE REGENERATION THROUGH SOX2-DEPENDENT SCHWANN CELL SORTING

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The peripheral nervous system has astonishing regenerative capabilities in that cut nerves are able to reconnect and re-establish their function. Schwann cells are important players in this process, during which they dedifferentiate en masse to a progenitor/stem cell and promote axonal regrowth. Here we report that fibroblasts also play a key role. Upon nerve cut, ephrinB-EphB2 signalling between fibroblasts and Schwann cells results in cell-sorting, followed by directional collective-cell migration of Schwann cells out of the nerve stumps to guide regrowing axons across the wound. Mechanistically, we show that cell-sorting downstream of EphB2 is mediated by the stemness factor Sox2 through N-cadherin relocalisation to Schwann cell-cell contacts. In vivo, inhibition or knockout of EphB2 impaired organised migration of Schwann cells, resulting in misdirected axonal regrowth. Our results identify a new link between Ephs and Sox proteins, providing a mechanism by which progenitor cells can translate environmental information to orchestrate the formation of new tissue.

IN VIVO IMAGING OF INJURY-INDUCED AXONAL RESPONSE IN THE MAMMALIAN SPINAL CORD

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A major hurdle in the study of spinal cord regeneration is the difficulty in positively identifying regenerating axons. Our lab has previously established a model for stable *in vivo* imaging of the injured spinal cord. This model has enabled us to examine axonal dynamics at a much higher spatial and temporal resolution than previously available. In this study, we used 2-photon imaging of dorsal column sensory axons to examine the acute and sub acute dynamics of axon degeneration/regeneration after axotomy via laser ablation. Because this model minimizes the confounding effects of scar tissue and other secondary injury responses as compared to conventional models of experimental spinal cord injury, it allows us to accurately investigate the intrinsic regenerative response of an axon after injury. We will present data testing whether the speed, the total distance of acute degeneration, or the anatomical location of axotomy in relation to the bifurcation point has any effect on the regenerative response days and weeks after axotomy. In addition, we will show that there are several forms of injury-induced regenerative growth: branching, elongation, and u-turn. These studies provide a platform to investigate the contribution of various intrinsic and extrinsic factors to injury-induced axonal growth.

A HOX NETWORK DEFINES RESPIRATORY MOTOR NEURON DEVELOPMENT.

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The diaphragm is the major respiratory muscle in mammals and its contraction is the final output of the activity of rhythmogenic respiratory networks in the brainstem. Motor neurons of the Phrenic Motor Column (PMC) provide the only motor neuron innervation to the diaphragm. Loss of function of PMC neurons is the leading cause of death in degenerative motor neuron diseases such as amyotrophic lateral sclerosis (ALS). Despite their critical role, the molecular mechanisms that control many aspects of PMC development like axonal trajectory, cell body clustering and intramuscular nerve branching remain largely unknown. We show that two Hox genes, Hoxa5 and Hoxc5, are critical for PMC development and that mice lacking both these Hox genes in motor neurons die at birth due to breathing defects. At the level of the spinal cord, PMC cell bodies appear reduced in numbers and are disorganized. Motor axons are still able to reach the diaphragm but once there fail to branch and form synapses on the entire muscle. In situ hybridization analysis reveals that the PMC of mutant mice shows a dramatic reduction in the expression of a number of proteins known to be involved in cell adhesion and axon guidance. Our data demonstrate a vital role for Hox5 proteins in PMC development and point to a general mechanism in which transcriptional regulation of column-specific genes by a Hox paralog group defines multiple aspects of motor neuron identity.

SEEING IS BELIEVING: IMAGING FUNCTIONAL CELL-CELL INTERACTIONS DURING PERIPHERAL NERVE DEGENERATION.

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Upon injury, the distal portion of a damaged peripheral nerve undergoes an active process of self destruction. This process of Wallerian degeneration is characterized by axonal fragmentation and subsequent debris clearance, which is mediated by Schwann cells and macrophages. Schwann cells present at the injury site are thought to provide essential signals that recruit macrophages, and macrophage infiltration and debris removal is considered a key prerequisite for axonal regeneration. Despite the significance of their proposed roles, when and how Schwann cells and macrophages interact with the injured nerve remains elusive, mainly due to the difficulties of continuously imaging the process of nerve degeneration in live, intact vertebrate animals. Similarly, the functional *in vivo* requirement of Schwann cells and macrophages in nerve degeneration and regeneration has yet to be defined.

Here, we report on a zebrafish model to define the interactions between injured peripheral nerves, Schwann cells, and macrophages *in vivo*. Using a laser mounted on a spinning disc confocal microscope we axotomize individual spinal motor nerves and image the entire process of axonal fragmentation and debris removal. We find that zebrafish axonal degeneration proceeds with the same morphological landmarks as previously reported for mammals. Minute-by-minute imaging reveals for the first time the dynamic changes preceding and during nerve fragmentation, with single axon resolution. Moreover, simultaneous live cell imaging of injured nerves and local cell types reveals that macrophages infiltrate the lesion site and contact the injured nerve prior to fragmentation, and that upon nerve fragmentation Schwann cells undergo rapid and extensive shape changes, while both cell types engulf axonal debris.

In mammals, over expression of the Wallerian Degeneration Slow (Wld^{s}) protein greatly delays nerve fragmentation, and static endpoint analyses suggest that these nerves fail to recruit macrophages, thereby delaying axonal regeneration. We find that the Wld^{s} protein also protects zebrafish peripheral nerves, indicating that the molecular mechanisms of axonal degeneration are conserved between mammals and zebrafish. Using live cell imaging we find that Wld^{s} expressing nerves efficiently recruit macrophages to the lesioned nerve, but that this is insufficient to induce axonal fragmentation. Moreover, using genetic ablations, we find that macrophages are dispensable for nerve degeneration, and that Schwann cells, thought to provide signals critical for macrophage recruitment, also appear dispensable for nerve degeneration. We will provide further data characterizing the role of Schwann cells and macrophages in peripheral nerve degeneration and regeneration.

THE HORMONE RECEPTORS HR51 AND E75 REGULATE AXON RE-EXTENSION FOLLOWING DEVELOPMENTAL PRUNING

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It has been well established that adult neurons in the CNS undergo little or no regeneration following insults such as spinal cord injury. In contrast, developing neurons are capable of extensive growth, extension and reorganization. Understanding the molecular mechanisms underlying neuronal plasticity during the course of normal development may therefore provide insights into the mechanisms that restrict regeneration of adult neurons. The neuronal remodeling events that occur during the development of the *Drosophila* mushroom body (MB) are initiated by axon pruning of γ-neurons, followed by axon re-extention to form adult specific connections. Currently, our knowledge of the molecular mechanisms underlying neuronal remodeling of γ-neurons remains fragmentary and in particular nothing is known about the axon re-extension phase. Only the γ-neurons in the developing MB undergo remodeling while others do not, offering a unique system in which axon re-growth can be distinguished from axon growth *per se*.

A MARCM forward genetic screen using *piggyBac* insertional mutagenesis has identified the orphan nuclear receptor, hormone receptor 51 (Hr51, also known as unf), as a key regulator of axon re-extension. MARCM analysis has shown that γ-neurons homozygous mutant for Hr51 extend axons normally in larvae, and undergo axon pruning at early pupa, yet fail to re-extend their axons to the adult specific connection. Later born neurons, also homozygous for the mutant allele, extend axons normally, indicating that the mutation affects axon re-extension and not axon growth *per se*. Genetic *in vivo* interaction experiments revealed that Hr51 can repress the expression of the nuclear receptor EcRB1, a known regulator of axon pruning. Interestingly, we also found that the nuclear receptor E75, which is known to be regulated by EcRB1, is required for axon re-extension. Because the mammalian orthologs of Hr51 and E75 (NR2E3 and NR1D1, respectively) were shown to function together in other systems, our data suggests that E75 and Hr51 may work together as heterodimers to induce axon regrowth following pruning. Our study demonstrates that the hormone receptors Hr51 and E75 may play a role in switching the growth status of axons from pruning to extension and that dynamic interactions between nuclear receptors may regulate different steps of neuronal remodeling. Understanding the mechanisms by which this network of nuclear receptors operates may provide insights into the mechanisms that restrict regeneration of adult neurons in the CNS.

REGION SPECIFIC PROJECTION BY TRIGEMINAL SENSORY NEURONS REQUIRES ROBO2.

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Trigeminal sensory neurons (TGNs) are the primary somatosensory neurons in the head to detect thermal, mechanical, and chemical stimuli. TGNs receive external stimuli via peripheral axons and propagate sensory information via central afferent axons to the hindbrain and spinal cord. Trigeminal central axons are roughly segregated based on sensory sensitivity, but it is unclear how afferent specificity is achieved during development and how precisely individual axons follow the modality rules. To address this question, we use zebrafish (*Danio rerio*) TGNs as a model system. Zebrafish larvae are small, transparent, and contain only ~60 TGNs per ganglion, making them ideal for live imaging. By using transgenic fish expressing various fluorescent proteins, we can observe central axon growth over time.

To test if TGNs with different sensitivity would have different afferent projection specificity, we performed single-cell labeling of two different TGN subsets. One labeled TGN subset expresses the nociceptive receptor, TRPA1B, important for sensing environmental chemicals; the other subset is TRPA1B negative, labeled by an Islet1 enhancer (Isl1SS). We found that both subsets innervate the rostral hindbrain, where they activate the Mauthner neurons that trigger rapid escape response. However, the TRPA1B population selectively innervates the caudal hindbrain, the region that is important for pain sensation in mammals.

To elucidate the molecular mechanisms that control the establishment of subset specific afferent projection, we examined the role of the Robo/Slit signaling pathway. First, we examined the expression of all Robo and Slit homologs in zebrafish. At the stage when TGN afferents are starting to form branches and synapses, two Robo (Robo1 and Robo2) are expressed in TGNs and two Slit (Slit1b and Slit3) are expressed adjacent to the TGN afferent. Robo1 is expressed at low levels in all TGNs. Surprisingly, Robo2 is preferentially expressed in the Isl1SS subset and absent in the TRPA1B subset. Consistent with a role for Robo2 in forming subset specific afferent projections, loss of Robo2 only affected the Isl1SS subset, increasing its innervation in the caudal hindbrain. These results suggest that Robo2 is required for TGN subset specific afferent projection.

In summary, our results showed that different TGN subsets have distinct afferent target areas and that Robo signaling is important for targeting specificity. Functionally, axon mis-targeting observed in Robo2 mutants may result in different behavioral responses initiated by nociceptive or tactile stimuli. We are currently testing this hypothesis with locomotor activity assays.

WNT-PLANAR CELL POLARITY SIGNALING CONTROLS THE ANTERIOR-POSTERIOR ORGANIZATION OF MONOAMINONERGIC AXONS IN THE BRAINSTEM

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Monoaminergic neurons (serotonergic (5HT) and dopaminergic (mdDA)) in the
brainstem project axons along the anterior-posterior (A-P) axis. Despite their important physiological functions and implications in disease, the molecular mechanisms that dictate the formation of these projections along the A-P axis in vivo remain poorly understood. Here we reveal a requirement for Wnt/PCP signaling in the A-P organization of both the 5HT and mdDA systems. We find that 5HT and mdDA axons express the core PCP components Frizzled3, Celsr3 and Vangl2. In addition, both ascending and descending projections show A-P guidance defects in Frizzled3, Celsr3 and Vangl2 mutant mice. The only known ligands for PCP signaling are Wnt proteins. Wnt5a and Wnt7b attract or repel 5HT and mdDA axons in vitro and are expressed in gradients along the A-P axis of the brainstem, consistent with their role as directional cues. In addition, Wnt5a mutants show transient A-P guidance defects in mdDA projections. We furthermore observe that the cell bodies of migrating descending 5HT neurons eventually re-orient along the direction of their axons. In Frizzled3 mutants, many 5HT and mdDA neuron cell bodies are oriented abnormally along the direction of their aberrant axon projections. This finding demonstrates that A-P axon guidance by Wnt/PCP signals is essential for the proper A-P cellular organization of monoaminergic nuclei, and this is likely to apply to other neural systems. In all, this study identifies Wnt/PCP signaling as a global A-P guidance mechanism that controls axonal and cellular organization beyond the spinal cord.

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MECP2 REGULATES MRNA TRANSLATION OF SYNAPTIC PROTEINS IN MOUSE BRAIN: IMPLICATION IN PATHOGENESIS OF RETT SYNDROME.

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MECP2 was identified as a Methyl-CpG DNA binding protein and is best known as a regulator of chromatin structure and transcription. Mutations in MeCP2 cause the autism spectrum disorder Rett syndrome (RTT), but the biological function of MeCP2 and pathogenic mechanism of RTT remain not well understood. Here we report a post-transcriptional role, including translation regulation, of MECP2. MECP2 is associated with ~1000 transcripts in mature mouse brain and directly binds mRNAs in-vitro. MECP2 is detected in poly-ribosomes of brain lysates and proteomic analysis indicate its interactions with multiple partners involved in mRNA transport and translation. In contrast to the subtle impact on steady-state transcript levels in the brain, MECP2 deficiency results in profound alterations in hundreds of actively translating mRNAs that are normally associated with MECP2 and their encoded proteins. Intriguingly, a significant number of MECP2-associated proteins as well as proteins encoded by MECP2 target transcripts are synaptic proteins. Our results establish a novel biological function of MeCP2 and implicate translation regulation, including translation of synaptic proteins, in the pathogenesis of RTT.

GENERATION OF AN EMBRYONIC CULTURE SYSTEM FOR THE INVESTIGATION OF STRIATAL MEDIUM SPINY NEURON DENDRITIC SPINE DEVELOPMENT AND PLASTICITY.

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Dendritic spines of Striatal Medium Spiny Neurons (MSNs) receive both dopaminergic inputs from the substantia nigra and ventral tegmental area and glutamatergic input from the cortex, thalamus, amygdala, and hippocampus. Experience-dependent structural plasticity of MSN dendritic spines has been reported under numerous circumstances, including repeated drug administration and disease states like Huntington's and Parkinson's. These structural changes can have functional consequences for entire circuits; however, the relationship between MSNs dendritic spine structure and function, as well as the molecular mechanisms regulating their development and plasticity are poorly described. One major difficulty faced when studying MSN development is the lack of an appropriate in vitro model system: unlike the hippocampal culture system, MSNs grown in striatal mono-cultures display stunted dendritic arborization and fail to develop a full cohort of mature dendritic spines.

Here we report the generation of a reliable, easy to reproduce embryonic mouse cortical-striatal co-culture system capable of promoting the survival and development of MSNs. Unlike MSNs in striatal mono-culture, MSNs in co-culture develop mature, in vivo-like morphologies, and have high densities of dendritic spines. Morphological identification of EGFP filled co-cultured MSNs can be confirmed by immunochemical detection of DARPP-32 (Dopamine and cyclic-AMP regulated phosphoprotein of 32 kDa). Additionally, co-cultured MSN spines contain PSD-95 puncta and are opposed to SV2 puncta, indicating the spines are morphologically mature. Finally, whole-cell recordings of co-cultured (versus mono-cultured) MSNs exhibit a significantly higher mEPSC frequency, which is consistent with an increase in synaptic contacts. These studies establish that our co-culture system is suitable for studying the molecular mechanisms that regulate the morphological and physiological development and function of MSN dendritic spines.

SIGNALING THROUGH NETRIN RECEPTORS PATTERNS MOTOR AXON TRAJECTORY IN THE DEVELOPING LIMB

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The formation of specific connections between motor neurons and their target muscles is essential for coordinated motor behavior. Axons from the lateral motor column (LMC) enter the limb, making sequential decisions – initially the selection of a dorsal or ventral limb mesenchyme trajectory, and later the choice of which muscles to innervate. Several ligand-receptor systems that control dorso-ventral LMC axon trajectory have been identified. Nevertheless, genetic evidence suggests that LMC neurons use additional molecules to achieve correct pathfinding. A screen for ligands and receptors expressed in the developing limb and by subsets of motor neurons led us to consider whether Netrin signaling is involved in the guidance of motor axons within the developing limb. We have found that Netrin-1 is expressed by cells of the dorsal limb mesenchyme, and that DCC, a receptor mediating Netrin-1 attraction, is expressed at high levels in neurons of the lateral division of the LMC that innervates the dorsal limb. In addition, Unc5c/Rcm, a receptor mediating Netrin-1 repulsion is expressed by medial LMC neurons that project to the ventral limb. Analysis of motor axon trajectories in *netrin-1, unc5c/rcm* and *dcc* mutant mice indicates that Netrin-1 acts as a bifunctional guidance cue, attracting lateral LMC axons and repelling medial LMC axons from the dorsal limb mesenchyme. Moreover, we have found that consistent with the mouse genetic analysis, in primary motor neuron cultures the axons of lateral LMC neurons grow preferentially on Netrin-1 stripes, whereas the axons of medial LMC neurons avoid Netrin-1 stripes.

Later, over the period of target muscle innervation, we find that Unc5c/Rcm expression resolves into a motor pool-specific pattern, and that in *unc5c/rcm* mutants, certain muscle nerve trajectories are disrupted. Together, these findings suggest that the dynamic profile of Netrin receptor expression by LMC neurons contributes to sequential aspects of motor axon pathfinding: the early establishment of dorso-ventral divisional branches and the later formation of muscle nerve trajectories. Netrin signaling therefore participates in the patterning of nerve-muscle connections in the mammalian limb, and we are now examining how distinct signaling systems are integrated by LMC growth cones to establish the pattern of nerve-muscle connectivity.

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COFILIN UNDER ARREST: A NEW ROLE FOR B-ARRESTINS IN CONTROLLING COFILIN ACTIVITY AND DENDRITIC SPINE REMODELING

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Dendritic spines are the postsynaptic sites of most excitatory synapses in the brain, and changes in their morphology are implicated in synaptic plasticity and long-term memory. F-actin dynamics are thought to be a basis for the formation of dendritic spines during development and their structural plasticity (Ethell and Pasquale, 2005; Pontrello and Ethell, 2009). We have previously shown that the F-actin-severing protein cofilin, which is regulated by phosphorylation, can induce remodeling of mature dendritic spines in hippocampal neurons (Shi et al., 2009). Our current studies demonstrate the dual regulation of cofilin by EphB and NMDA receptors in mature dendritic spines through modulation of two competing pathways, CaMKII-mediated suppression of cofilin activity by phosphorylation, and calcineurin-dependent cofilin activation through dephosphorylation. These pathways control the delicate balance between the maintenance of mature dendritic spines and their remodeling. While EphB receptors suppress cofilin activity through LIMK-mediated cofilin phosphorylation under normal synaptic activity, NMDAR activation triggers calcineurin-mediated activation of slingshot, which counteracts the phosphorylation of cofilin by LIMK1, causing an overall decrease in p-cofilin levels and cofilin activation. Interestingly, in response to NMDAR activation, EphB receptors potentiate NMDAR-mediated cofilin dephosphorylation and activation. NMDA receptor activation also promotes translocation of cofilin to dendritic spines, a process that requires cofilin dephosphorylation. However, cofilin dephosphorylation is not sufficient to trigger cofilin clustering in dendritic spines, an event that is also dependent on β-Arrestins. While β-Arrestins were first identified as mediators of G-protein coupled receptor signaling, they were recently suggested to regulate cofilin activity/ localization through scaffolding cofilin with enzymes that regulate its activity. Our studies demonstrate that NMDA-mediated translocation of cofilin into the spines is affected in β-Arrestin2-deficient neurons. While β-Arrestin2 is involved in NMDAR-dependent re-distribution of cofilin and its accumulation in dendritic spines, cofilin localization is also regulated by β-Arrestin1 under normal synaptic activity. Future studies will continue to investigate mechanisms that are involved in the regulation of cofilin activity and localization in dendritic spines, and their roles in dendritic spine development and remodeling.

KIRREL FAMILY MEMBERS IN THE DEVELOPMENT OF THE ACCESSORY OLFACTORY SYSTEM

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The olfactory systems play a critical role in the survival and mating behavior of most terrestrial vertebrates. Two different classes of odorants, general odorants and pheromones, are processed by the olfactory systems and convey several cues in vertebrates such as the presence of danger or of food, as well as social and sexual cues. In both the main and accessory olfactory systems, axons of chemosensory neurons must converge in the olfactory bulbs and form stereotypic connections with second order neurons in structures termed glomeruli. The proper convergence of olfactory axons into glomeruli and the formation of these stereotyped connections are essential for olfactory function. In the main olfactory system, the Kirrel family members have been implicated as homophilic adhesive molecules that play an important role in axon-axon interactions[4]. These interactions have been suggested to regulate olfactory nerve fasciculation and olfactory convergence; both crucial features of accurate glomerular map formation. In contrast to the main olfactory system, the molecular mechanisms that regulate glomerular axonal convergence in the accessory olfactory system are poorly understood. To begin to investigate the role of the Kirrel family members in the development of the accessory olfactory system, we have performed a detailed analysis of the spatio-temporal patterns of expression of these molecules by *in situ* hybridization and immunohistochemistry in the vomeronasal organ and the accessory olfactory bulb.

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OLIGODENDROCYTE-MYELIN GLYCOPROTEIN AND NOGO NEGATIVELY REGULATE ACTIVITY-DEPENDENT SYNAPTIC PLASTICITY

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In the adult mammalian CNS, the growth inhibitors oligodendrocyte-myelin glycoprotein (OMgp) and Nogo are broadly expressed in oligodendrocytes and neurons. Nogo and OMgp complex with the neuronal cell surface receptors Nogo receptor-1 (NgR1) and paired immunoglobulin-like receptor B (PirB) to regulate neuronal morphology. In the healthy CNS, components of the NgR1 complex have been implicated in activity-dependent refinement of neuronal connectivity. In the visual system, *NgR1*, *Nogo-A/B* and *PirB* participate in the consolidation of neuronal connectivity established during the critical period (McGee et al., 2005, Syken et al., 2005) and in the hippocampus, *NgR1* and *p75* regulate dendritic spine morphology (Zagrebelsky et al., 2005; Lee et al., 2008). *NgR1* also limits activity-dependent synaptic strength at Schaffer collateral-CA1 synapses (Lee et al., 2008), and down-regulation of NgR1 expression is required for consolidation of long-term spatial memory (Karlén et al., 2009). Here we examine whether Nogo and OMgp influence functional synaptic plasticity, the efficacy by which synaptic transmission occurs. In acute hippocampal slices, Nogo-66 and OMgp suppress NMDAR-dependent long-term potentiation (LTP) when locally applied to Schaffer collateral-CA1 synapses. *PirB-/-* and *NgR1-/-* single mutants and *NgR1-/-;PirB-/-* double mutants show normal LTP, indistinguishable from wild-type controls. Whereas, LTD in *NgR1-/-* but not *PirB-/-* mice is absent. Mechanistic studies revealed that Nogo-66 and OMgp suppress LTP in an NgR1–dependent manner. OMgp inhibits LTP in part through *PirB* but independently of *p75*. This suggests that NgR1 and PirB participate in ligand-dependent inhibition of synaptic plasticity. Moreover, loss of *NgR1 in vivo* leads to increased phosphorylation of erk1/2, signaling intermediates known to regulate neuronal growth and synaptic function. In primary hippocampal neurons, phosphorylation of AKT and p70S6-kinase is attenuated in the presence of myelin inhibitors. Collectively, we provide evidence that mechanisms of neuronal growth inhibition and inhibition of synaptic strength are related. Thus, myelin inhibitors and their receptors may coordinate structural and functional neuronal plasticity in CNS physiology and disease.

THE ROLE OF CLASS 4 SEMAPHORINS IN INHIBITORY SYNAPTIC DEVELOPMENT

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The molecular mechanisms that underlie inhibitory synapse development in the mammalian central nervous system remain to be elucidated. We discovered that RNAi mediated knockdown of a class 4 semaphorin family member, Sema4D, leads to a significant decrease in inhibitory synapse density in hippocampal neurons. This suggests that an important function of Sema4D is to promote inhibitory synapse formation. Interestingly, the transmembrane protein Sema4D is cleaved in lymphocytes (Elhabazi et al, *J Immunology*. 2001 166:4341) and platelets to specifically regulate platelet aggregation and thrombolytic formation (Zhu et al, *PNAS*. 2007 104:1621). We have demonstrated that Sema4D is cleaved in neurons. We hypothesize that this proteolytic cleavage is a metalloprotease-dependent event and are in the process of identifying which matrix metalloprotease family member is responsible for Sema4D cleavage by using a combination of chemical inhibitors and RNAi based approaches. Further, we seek to determine whether or not proteolytic cleavage may influence how Sema4D elicits its effects on inhibitory synaptic development. To achieve this goal, we have constructed multiple chimeras of Sema4D, including a non-cleavable form, by replacing various domains of Sema4D with that of the transmembrane protein CD4, a small single pass protein involved in T-cell activation. We plan to observe whether or not these chimeric mutants can rescue a decrease in inhibitory synaptic density in neurons that have undergone Sema4D knockdown. Our goal is to use these chimeric mutants as well as our understanding of proteolytic processing of Sema4D to determine the mechanism by which Sema4D effects inhibitory synapse formation.

NETRIN-1 BINDING ON APP CONTRIBUTES TO COMMISSURAL AXON GUIDANCE.

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The b-amyloid precursor protein (APP) is an orphan transmembrane receptor whose physiological role is largely unknown. APP is cleaved by proteases generating amyloid-β (Aβ) peptide, the main component of the amyloid plaques that are associated with Alzheimer's disease. However we have recently shown that APP binds netrin-1, the multifunctional guidance cue. We have shown that netrin-1 binding modulates APP signaling triggering APP intracellular domain (AICD)-dependent gene transcription. Furthermore, netrin-1 binding suppresses Aβ peptide production in brain slices from Alzheimer model transgenic mice. In this mouse model decreased netrin-1 expression is associated with increased Aβ concentration, thus supporting netrin-1 as a key regulator of Aβ production. However, while the significance of netrin-1 interaction with APP in pathological condition may appear obvious, one important question remains to understand the role of this interaction in the "classic" axon guidance function of netrin-1. We will show here that APP as a receptor for netrin-1 and as a co-receptor for DCC is an important component of the receptor complex that mediates netrin-1-induced commissural axon guidance.

NR-CAM AND PLEXIN-A1 IMPLEMENT PROPER CROSSING AT THE OPTIC CHIASM AND TARGETING IN THE DLGN

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A direct consequence of retinal axon decussation in the optic chiasm is eye-specific innervation of the dorsal lateral geniculate nucleus (dLGN). Analysis of retinogeniculate projections in mouse models in which divergence at the optic chiasm is perturbed (e.g., EphB1$^{-/-}$ mice, Rebsam et al., J. Neurosci., 2009), provides an opportunity to address the coordinate action of molecular cues and neural activity in the formation of eye-specific innervation and retinotopy in visual targets. In other work from our lab, we found that Nr-CAM, Plexin-A1 and Semaphorin6D act in concert for proper chiasm formation and the appropriate balance of ipsi-contralateral projections (see Kuwajima et al., this meeting). In Sema6D$^{-/-}$ and Plexin-A1$^{-/-}$;Nr-CAM$^{-/-}$ mutants, RGC axons with a crossed trajectory defasciculate at the optic chiasm and more readily project ipsilaterally. In normal patterns of decussation at the chiasm, retinal fibers fasciculate in the chiasm with fibers from their own and opposite eye, and in the optic tract in topographic order, but whether these specific patterns of fasciculation are necessary for innervation of visual targets is not understood. In the dLGN of Plexin-A1$^{-/-}$;Nr-CAM$^{-/-}$ mutants, RGC axons from both ipsi- and contralateral eyes misproject to ectopic locations separated from the rest of the dLGN by the optic tract and form aberrant eye-specific patches. While Nr-CAM, Plexin-A1 and Sema6D are expressed in the dLGN, Nr-CAM single mutants do not show this phenotype. Analyses of RGC projections in the Plexin-A1$^{-/-}$ dLGN is in progress as is more refined retinal labeling to identify the source of the misprojections.
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REWIRING THE MOUSE FACIAL SOMATOSENSORY MAP

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In vertebrates, the central nervous system receives and processes somatosensory inputs from the opposite side of the body. Whether this organization has a functional role in the processing of the information coming from the periphery is unclear. Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS) is an inherited disease in which sensory inputs and motor outputs of the cortex are uncrossed. Intriguingly these patients do not exhibit strong sensory or motor deficits, except the horizontal conjugate eye palsy. How the cortex reorganizes and how the plasticity of the network compensates the anatomical defects is unknown. All HGPPS patients carry mutations in the ROBO3 gene which encodes a receptor required for midline crossing by commissural axons in the hindbrain and spinal cord of vertebrates. We generated a Robo3 conditional knock-out and showed (Renier et al 2010) that it can be used in combination with specific CRE lines, to force hindbrain commissural axons to project to their target cells but ipsilaterally, on the wrong side of the brain.
The trigeminal nucleus principalis (PrV) is located in rhombomere 3 and receives inputs from the whiskers. PrV neurons project to the contralateral ventrobasal thalamus (VB) where the information is relayed to the somatosensory cortex (S1). Genetic fate mapping has shown that PrV neurons originate only from rhombomere 3. To try to recapitulate the HGPPS condition, and study the consequence of ipsilateral rewiring of somatosensory information on cortex organization, we forced PrV neurons to project to the ipsilateral thalamus by crossing Robo3lox/lox mice to Krox20:cre transgenics (where cre is only expressed in r3 and r5)
We show here that in krox20:cre;Robo3lox/lox mice most PrV to VB axons are uncrossed. Most intriguingly, a disrupted organization of the whisker representation is noted in the VB and the cerebral cortex, with supernumerary barreloids and barrels. Unilateral stimulation of the whiskers resulted in a bilateral activation of the barrel cortex in mutants, contrasting with the purely contralateral activation in controls. This lead us to propose the existence of 2 non-overlapping maps, one activated by the contralateral inputs and one by the ipsilateral inputs. Further experiments are in progress to test this hypothesis. We are now trying to determine if these defects duplication of sensory inputs perturbs tactile behaviour such as whisking.

CHARACTERIZING THE ROLE OF WNT-RYK SIGNALING IN MEDIOLATERAL TOPOGRAPHIC MAPPING AND TARGET INNERVATION OF RETINOTECTAL CONNECTIONS

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Topographic mapping requires opposing forces that direct the termination of axons to the correct topographic positions. We proposed that Wnt3-Ryk signaling counterbalances that of ephrinB1-EphB along the tectal mediolateral axis in chick retinotectal mapping (Schmitt et al., 2006). To determine how these counterbalancing forces interact *in vivo*, we studied the expression of these receptors in chick retinal ganglion cells (RGCs) and then manipulated receptor levels of Ryk and EphB2 via *in ovo* electroporation. We observed that Ryk and EphBs are localized in the same RGC growth cones and axons in retinal culture, suggesting that they may trigger competing signals in the same growth cones or branches. RGC axons produce interstitial branches that are directed medially or laterally, and these branches then innervate the retinorecipient layers to make final synaptic connections in the correct topographic positions. We found overexpression of Ryk and EphB2 in RGCs produced pronounced opposite shifts in interstitial branch direction in the tectum, with Ryk overexpression promoting laterally-directed branches while EphB2 overexpression resulted in more medially-directed branches. We are currently determining whether the bias of branch directions occurs at the initiation of branching, during the growth of the branches, or by selective pruning. From the interstitial branches, RGC axons further branch into more complex arbors, which then make connections with tectal target cells. Both Ryk and EphB2 appear to affect the development of interstitial branches, with Ryk promoting branch elongation while EphB2 increases further branching. In addition, the retinotectal system appears to develop in a medial-to-lateral temporal order, suggesting a temporal program in the maturation of the map. We are currently testing the role of Wnt-Ryk signaling in tectal innervation by RGC axons.

Schmitt, A.M., Shi, J., Wolf, A.M., Lu, C.C., King, L.A., and Zou, Y. (2006). Wnt-Ryk signalling mediates medial-lateral retinotectal topographic mapping. Nature *439*, 31-37.

A ROLE FOR VAV GEFs IN EPHB1-DEPENDENT IPSILATERAL AXON TRACT FORMATION

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Retinal ganglion cell (RGC) axons decussate during embryonic development at the optic chiasm (OX). RGC axons derived from the ventrotemporal retina express EphB1 Receptors, and these axons repel from glial-expressed ephrinB2 at the OX midline to form the ipsilateral tract. Previously, we found that knockout mice lacking Vav family Rho GTPase guanine nucleotide exchange factors (GEFs) had decreased ipsilateral axon terminals in the dorsal lateral geniculate nucleus (dLGN), suggesting a potential role for Vav GEFs in EphB1 forward repulsive signaling. We now find that Vav-deficient mice have significantly decreased ipsi axons at a time just after they repel from the OX, consistent with a defect in midline repulsion in these Vav2/3 null mice. Consistent with these observations, we find that clustered ephrinB2 stimulates Vav2 tyrosine phosphorylation in cultured primary neurons, and co-expression of EphB1 with Vav2 results in increased levels of Vav2 phosphorylation at P-Y172, a key residue that regulates Vav2 RhoGEF function. Interestingly, expression of a dominant negative Dynamin (K44A) or treatment with an putative endocytosis inhibitor, PAO, dramatically reduces ephrinB2-induced growth cone collapse in cultured neurons, suggesting that EphB Receptor internalization, which is possibly regulated by Vav, is an important process for ephrinB2-induced growth cone repulsion. While still preliminary, these findings suggest that EphB1 forward signaling may regulate repulsion of RGC axons at the OX by activating Vav GEF activity and EphB Receptor endocytosis.

KINESIN-2 AND +TIPS ARE REQUIRED FOR UNIFORM DENDRITE MICROTUBULE POLARITY AND FOR REGENERATION OF AXONS FROM DENDRITES

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Axons and dendrites have different functions, components, and cytoskeletal organizations. One of the most striking differences between axons and dendrites of Drosophila neurons is the opposite polarity of microtubules in these two compartments. Mechanisms that control uniform minus-end-out dendritic microtubule polarity have not previously been identified. Using live imaging of neurons in vivo, we find that dendrite microtubules are dynamic and often grow through branch points. Moreover their growth through branch points is directed: 98% of the time they grow towards the cell body. This allows the cells to maintain uniform microtubule polarity in the face of constant microtubule remodeling. Two-color live imaging of growing microtubule plus ends and stable microtubules allowed us to determine that growing microtubules use stable microtubules as tracks to guide them through branches. We hypothesized that this tracking would involve a kinesin attached to the microtubule plus end with +TIPs. In a candidate screen we found that kinesin-2 and the +TIPs EB1 and APC are required for uniform dendrite microtubule polarity. Moreover, the protein-protein interactions and localization of Apc2-GFP to branch points suggests these proteins can work together at dendrite branches. We propose that kinesin-2 is recruited to growing microtubules by +TIPS, and that the motor protein steers growing microtubules towards the cell body at branch points. This represents a new mechanism to maintain polarized arrays of microtubules. We demonstrate the importance of this mechanism by showing that axon regeneration from a dendrite after complete axotomy requires kinesin-2.

HYDROGEN PEROXIDE PROMOTES PERIPHERAL SENSORY AXON REGENERATION IN WOUNDED ZEBRAFISH EPIDERMIS

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Functional recovery from epidermal injury requires innervation of healing skin by somatosensory axons. Using zebrafish larval fin amputation as a model, we investigated the influence of epidermal injury on peripheral sensory axon regeneration. At larval stages, when precisely severed, regenerating axons are normally not capable of reinnervating denervated epidermis, we amputated the caudal fin and used time-lapse confocal imaging to visualize the behavior of injured GFP-expressing somatosensory axons over 12h. Somatosensory axons always regenerated into the wound epidermis, suggesting that fin injury can overcome growth inhibitors normally present at this stage. Injury promoted axon growth only when the injured axon was close to the amputation site, implying that the signal from damaged skin functions at short range. To identify the source of regeneration signals, we laser damaged a few keratinocytes; ablating keratinocytes in the tail or the head was sufficient to promote robust sensory axon growth near the wound. We next tested the possibility that H_2O_2 mediated injury-dependent axon regeneration, since high concentrations of this reactive oxygen species were recently shown to be produced along the wound margins of injured zebrafish fins. Indeed, the addition of H_2O_2 to the media promoted peripheral sensory axon growth and regeneration following axotomy in uninjured fins. Conversely, inhibition of H_2O_2 production via morpholino knockdown of the dual oxidase 1 (*duox1*) gene completely prevented axon growth following fin injury. H_2O_2 is also known to promote leukocyte homing to wounds in the zebrafish fin, but H_2O_2 was still capable of promoting axon growth in the absence of blood cells, demonstrating that its roles in leukocyte recruitment and axon growth are separable. Together these findings suggest a novel function for H_2O_2 in coordinating the regeneration of skin with the axons that innervate it.

ROBO MEDIATED INHIBITION OF N-CADHERIN ADHESION: A MECHANISM FOR GUIDING POST-CROSSING COMMISSURAL AXONS INTO LONGITUDINAL TRACTS AND TO CENTRAL TARGETS

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Dorsal spinal commissural (DSC) neurons are a well-studied model system for investigating the molecular logic of axon pathfinding at and beyond the CNS midline. Despite significant progress in elucidating the mechanisms that direct commissural axons to the floor plate (FP) at the ventral midline of the spinal cord, we do not have a clear understanding of the contralateral navigational program, which guides decussated axons into appropriate longitudinal tracts and to their poorly characterized synaptic targets. To selectively visualize contralateral projections elaborated by commissural neurons, we analyzed the trajectory of genetically distinct dI2 DSC neurons by using in ovo electroporation to unilaterally transfect a reporter construct harboring a *Neurog1* enhancer element, which is specific for dI2 progenitors, in chick embryos. Most labeled, decussated dI2 commissural axons project away from the ventral midline along rostrally directed sigmoidally shaped trajectories. Although it has been suggested that dI2 axons contribute to the spinothalamic tract, we find that these axons project to the developing cerebellum, hindbrain, and midbrain. Notably however, the mechanisms that guide dI2 axons into longtiudinally-oriented ascending tracts, which project over long distances to these central targets, remain to be defined. We have previously shown that, as a consequence of disabling Robo-Slit signaling in chick embryos most decussated dI2 axons project alongside, rather than extending away from, the ventral midline of the spinal cord. Given that Robo can inhibit N-cadherin mediated cell adhesion in vitro (Rhee et al., 2002), we asked whether the post-crossing phenotype observed in the absence of Robo-Slit signaling reflects the inability of Robo to abrogate N-cadherin mediated hyperfasciculation of post-crossing commissural axons. Consistent with this hypothesis, most decussated dI2 axons that mis-express high levels of N-cadherin project alongside, instead of projecting away from, the floor plate. Moreover, morpholino-mediated knockdown of N-cadherin expression or disabling N-cadherin function through the expression of a dominant-negative form of the protein, rescues the dominant negative Robo phenotype. Our studies show, for the first time, that crosstalk between Robo and N-cadherin regulates the guidance of post-crossing commissural axons by modulating their fasciculation, and likely facilitates their projection to appropriate central targets.

ACTIVITY-DEPENDENT MODULATION OF SURFACE LOCALIZATION OF FRIZZLED-5, A RECEPTOR FOR THE SYNAPTIC ORGANIZER WNT7A

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Wnt proteins play a critical role in several aspects of neuronal circuit formation. Wnts can signal through different receptors including Frizzled, Ryk and Ror2. In the hippocampus, Wnt7a is required for the formation of synapses, however its receptor remains poorly characterized. Here, we demonstrate that the Frizzled-5 receptor (Fz5) is expressed during the peak of synaptogenesis in the mouse hippocampus. Surface Fz5 is present at pre and postsynaptic sites. Gain of function of Fz5 during early stages of synaptogenesis increases the number of presynaptic sites in hippocampal neurons. Conversely, Fz5 knockdown or the soluble Fz5-CRD domain (Fz5CRD), which binds to Wnt7a, block the ability of Wnt7a to stimulate synaptogenesis.

To begin to understand the mechanisms that regulate the localization of Fz5 at synapses, we investigated the role of neuronal activity, which is known to regulate the localization of TrkB and glutamate receptors. Increased neuronal activity induced by K+ depolarization or by high frequency stimulation (HFS), known to promote synapse formation, raises the levels of Fz5 at the cell surface. Importantly, both stimuli increase the localization of Fz5 at synapses, an effect that is blocked by Wnt antagonists or by Fz5CRD, a soluble domain of Fz5. Conversely, low frequency stimulation, which reduces the number of synapses, decreases the levels of surface Fz5 and the percentage of synapses containing this receptor. Crucially, Fz5CRD abolishes HFS-induced synapse formation. Our results demonstrate that Fz5 mediates the synaptogenic effect of Wnt7a and that its localization to synapses is regulated by neuronal activity, a process that depends on endogenous Wnts. These findings support a model where neuronal activity and Wnts increase the responsiveness of neurons to Wnt signalling by recruiting Fz5 receptor at synaptic sites. Moreover, our results demonstrate that Wnt-Fz5 signalling is critical for neuronal activity mediated synapse formation.

ROLE FOR FRIZZLED 3 AND CELSR 3 IN ENTERIC NERVOUS SYSTEM PATTERNING

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The enteric nervous system (ENS) is the largest compartment of the peripheral nervous system which regulates intestinal motility, secretion and blood flow. The mature ENS consists of many distinct subtypes of neurons which express specific molecular markers and have characteristic morphology, axonal projection patterns and function. Correct connectivity of these neurons is necessary for the development of normal gastrointestinal motility. In mice, the wiring of the ENS takes place over several embryonic days and it is thought to be completed at postnatal stages, when coordinated motility patterns can be detected. Currently, very little is known about the mechanisms by which the axons and dendrites of enteric neurons are guided to their correct targets to form functional circuits.

To provide insight into the mechanisms that control wiring of the ENS, we have analysed the role of the Wnt receptor Frizzled 3 (Fzd3) and the core component of the Planar Cell Polarity pathway Cadherin EGF LAG seven-pass G-type receptor 3 (Celsr3). These molecules have been shown to be important for the development of major neuronal tracts in the CNS and, interestingly, they are both expressed in a subset of progenitors/newly differentiated neurons in the developing ENS. Mice deficient in either protein had characteristic defects in the projections of enteric neurons which were evident from early stages of ENS development. Immunostaining with pan-neuronal markers and DiI labeling of neuronal projections revealed clear deficits in axonal tracts and the organisation of neuronal processes within the gut. In addition, by labeling individual neurons and their projections in the gut of mutant embryos, we have uncovered characteristic abnormalities in the morphology and projection pattern of enteric neurons. To examine the functional implications of these defects, we have used a conditional knock-out allele of *Celsr3* to specifically delete the gene from the neural crest cell lineage. After weaning Celsr3|Wnt1 animals showed reduced survival and a consistent decreased in body weight. Anatomical inspection of the intestine often revealed obstruction and abnormally contracted and relaxed segments consistent with defects in intestinal motility. Our data establish for the first time a role for *Fzd3* and *Celsr3* in ENS development and provide novel insight into the molecular pathways important for connectivity of enteric neurons.

THALAMUS-DERIVED MOLECULES PROMOTE SURVIVAL AND DENDRITIC GROWTH OF DEVELOPING CORTICAL NEURONS

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During development, intrinsic and extrinsic factors cooperate to regulate cell differentiation. It has been suggested that an afferent-derived factor is involved in cortical development as an extrinsic factor. However, the molecular mechanism is almost unknown. Here, we attempted to identify the molecules that are expressed in sensory thalamic nuclei and affect cortical cell differentiation. First, thalamus-specific molecules were searched by constructing a subtraction cDNA library which is enriched for genes expressed in the thalamus but not in cortex of newborn rats, and by comparing gene expression profiles of the thalamus and cortex with DNA arrays. A systematic screening with *in situ* hybridization showed that several genes encoding extracellular molecules were strongly expressed in sensory thalamic nuclei at early postnatal stages. Transfection of genes encoding their tagged proteins into cultured thalamic neurons demonstrated that only two extracellular molecules, Neuritin-1 (NRN1) and VGF, were transported to axon terminals, which is a necessary condition for thalamic afferent-derived factors. Furthermore, the effects of these proteins on cellular differentiation were studied using dissociated cortical cell culture. Addition of NRN1 and VGF to the culture medium increased the number of survived neurons after one week *in vitro*. The number and length of dendrites were also increased significantly. Taken together, these results suggest that NRN1 and VGF are strongly expressed in developing sensory thalamic neurons, and promote cell survival and dendritic growth of cortical neurons.

CGMP SIGNALING REGULATES BIFURCATION OF SENSORY NEURONS.

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Axonal branching - either by growth cone bifurcation or by sprouting of collaterals from the axon shaft - substantially adds to the complexity of neuronal circuits. Our studies on dorsal root ganglion (DRG) neurons identified a cGMP signaling cascade - comprising the receptor guanylyl cyclase Npr2 and the cGMP-dependent protein kinase I (cGKI) - essential for bifurcational branching of sensory afferent projections at the dorsal root entry zone (DREZ) of the spinal cord. Recently, we demonstrated that the secreted protein C-type natriuretic peptide (CNP) is expressed in the dorsal cord when sensory axons arrive at the DREZ and activates cGMP signaling in DRG via Npr2. Like Npr2 and cGKI mutant mice the CNP knock-out shows a complete lack of sensory axon bifurcation at the DREZ. Functionally, the disturbed axonal bifurcation in mutants of cGMP signaling components is accompanied by reduced synaptic input onto second-order neurons in the dorsal horn of the spinal cord.
To complement our understanding of cGMP signaling evoked axon bifurcation we searched for phosphorylation targets of cGKI in DRG. Using phospho-specific antibodies specific for the consensus phosphorylation site of cGKI we detected various phosphorylation targets of cGKI in DRG lysates after stimulation with analogs of cGMP. So far our analysis of genetic mouse models for the identified phosphorylation targets could not exclude the possibility that an orchestrated effort of several components mediates sensory axon bifurcation.
Cytosolic cGMP becomes hydrolyzed by phosphodiesterases (PDEs). Some PDEs possess a dual specificity towards the degradation of both cGMP and cAMP enabling a cross-talk of these two second messengers. An RT-PCR screen followed by in situ hybridization identified a cGMP/cAMP degrading PDE selectively expressed in embryonic DRGs. Biochemical studies using embryonic DRGs demonstrate that this PDE is responsible for the degradation of cGMP generated after stimulation by CNP. Analysis of a PDE knock-out model does not reveal bifurcation errors at embryonic stages excluding an involvement of cAMP at this level.
The complexity of cGMP signaling is further increased through the scavenger receptor Npr3 that lacks an intracellular guanylyl cyclase domain. We observed that Npr3 is selectively expressed at the dorsal roots. Its absence results in bifurcation errors of only a small percentage of sensory axons suggesting that the availability or the concentration of CNP at appropriate places is essential for correct axon bifurcation.
Hence, our data demonstrate that additional components modulate the CNP-Npr2-cGKI signaling axis that triggers sensory axon bifurcation.

AMPA RECEPTOR DYSFUNCTION, SPINE MATURATION ALTERATION AND INCREASED SUSCEPTIBILITY TO APOPTOSIS IN THE HIPPOCAMPUS OF THE MOUSE MODEL OF COFFIN-LOWRY SYNDROME (CLS)

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The CLS is a handicapping syndromic form of X-linked mental retardation, caused by loss of function mutations in the gene RPS6KA3 coding for RSK2. Affected patients develop facial abnormalities, skeletal deformations and psychomotor retardation. RSK2 belongs to a family of 4 highly homologous members. RSKs are serine-threonine kinase acting at the end of the Ras/MAPK signalling pathway by phosphorylating various cytoplasmic and nuclear substrates. RSKs are implicated in important cellular events, including proliferation, cell survival and neuronal outgrowth. RSK2-KO mice exhibit behavioural abnormalities, including impaired spatial learning and memory. Moreover, in the adult mouse brain, RSK2 is highly expressed in the hippocampus, a structure involved in these cognitive processes.
In order to unravel the molecular and cellular mechanism underlying cognitive dysfunction in RSK2-KO mice and patients, we are investigating the mechanisms involved in cell survival, neurotransmission and synaptic plasticity in the hippocampus.
In the current study, comparison of hippocampal gene expression profiles from RSK2-KO and WT littermate mice revealed differential expression of 100 genes encoding proteins acting in various biological pathways. One third of the dysregulated genes have been implicated in cell death and we show that RSK2-KO primary hippocampal neurons are significantly more susceptible to apoptosis than WT neurons. One up-regulated gene (Gria2) encodes the subunit GluR2 of the AMPA receptor. We provide evidence that the expression of GluR2 is significantly increased in the hippocampus of RSK2-KO mice and at synapses in KO hippocampal cultures, whereas basal AMPA receptor-mediated transmission is significantly decreased. Moreover we also found an increased density of mature dendritic spines along KO neuritis, but fewer excitatory synapses than in WT neurons. This alteration of spines maturation may be linked to the hyperactivation of the Ras/MAPK pathway in RSK2-KO neurons. Indeed we observed, in these cells, an increase of ERK phosphorylation, resulting in an alteration of transcriptional activity and in a modification of the actin polymerisation state.
Our findings suggest that loss of neurons due to increased incidence of apoptosis and a defect in AMPA neurotransmission and in synaptic plasticity mechanisms contribute to mental retardation in CLS patients.

THE IGCAM CAR (COXSACKIEVIRUS ADENOVIRUS RECEPTOR) ESTABLISHES A LINK BETWEEN NEURONAL ACTIVITY AND CELL CELL ADHESION

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Neuronal activity plays essential roles in all developmental stages. Here we have asked whether 1) neuronal activity regulates surface expression or complex formation of IgCAMs or whether 2) IgCAMs itself modulate neuronal activity.
Regulation of cell surface expression of IgCAMs by neuronal activity might occur through several processes including endocytosis, exocytosis or proteolysis. Modulation of surface expression or clustering might then affect cell-cell communication. We studied surface expression and complex formation of IgCAMs (NCAM, L1, Nf, NrCAM, CHL1, TAG-1, F11, Ntra, LAMP, CAR) after depolarization in intact embryonic tecta and analysed recycling of these proteins. In these biochemical assays we did not observe any significant changes of IgCAMs after KCl treatment.
We then tested whether IgCAMs itself modulate electric activity by analysing action potential generation in cell cultures of several knock-out mice of IgCAMs. We observed that in the absence of CAR the frequency of action potentials induced by depolarizing currents is enhanced. While the current densities of voltage-gated Na^+ and K^+ currents are not changed, we analysed the electrotonic membrane properties which may affect the firing pattern. Interestingly, the input resistance (R_{IN}) is selectively increased in CAR deficient neurons only, while other IgCAM- knockout neurons did not reveal these changes, suggesting that CAR may modulate electric activity of neurons. Similarly, cardiomyocytes which also express CAR and which are excitable cells reveal a higher beat frequency in the absence of CAR.
To test whether these observations relate to cell cell adhesion we used the fiber knob of the adenovirus - a homotrimeric protein that binds up to three CAR polypeptides and which interferes with cell cell contact formation. This CAR binding reagent induced a decrease of the frequency of action potentials as a result of a reduced R_{IN}. On the basis of several electrophysiological recordings on ion conductance's we concluded, that the changes in R_{IN} in the absence of CAR results from an impaired Cl^- conductance, while treatment with fiber knob enhances a Cl^- conductance. The long-term consequences of the absence of CAR lead to modified electrotonic membrane properties resulting in a change in firing pattern and in an imbalance of inhibitory and excitatory input ratio in culture.
In summary, our observations indicate that CAR - in contrast to other IgCAMs - is able to modulate neuronal activity. It might contribute to a fine tuning of electric activity within neighbouring groups of cells during developmental periods.

A MOLECULAR MECHANISM FOR LATERAL POSITIONING OF DIENCEPHALOSPINAL LONGITUDINAL AXONS: NEGATIVE CONTROL OF ROBO3 BY BHLH-PAS TRANSCRIPTION FACTORS SIM1A AND ARNT2 FACILITATES REPULSION THROUGH ROBO2

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The two bHLH-PAS transcription factors Sim1a and Arnt2 are required for differentiation of dopaminergic and neuroendocrine neurons in the ventral diencephalon in zebrafish. Particular subsets of these Sim1a/Arnt2 dependent neurons establish diencephalospinal projection tracts, thought to be involved in control of motor activity patterns.
Here we report the roles of Sim1a and Arnt2 during diencephalospinal longitudinal tract formation. Depletion of *sim1a* or *arnt2* function by injecting antisense morpholino oligonucletides leads to midline shifting of longitudinal diencephalospinal axons, revealing that *sim1a* and *arnt2* are necessary for lateral positioning. Analyzing expression of the two known zebrafish *robo3* isoforms, we found increased expression of *robo3v2* in the ventral diencephalon after knockdown of *sim1a* or *arnt2*. Furthermore, we show that midline shifting of longitudinal axons is absent after *sim1a* or *arnt2* loss of function in *robo3* mutant background. Taken together, these data provide evidence for negative transcriptional control of *robo3v2* by Sim1a and Arnt2.
To test whether ectopic Robo3v2 interferes with Robo2 function, which is required for lateral positioning of diencephalospinal axon tracts, we generated *robo2; robo3* compound mutants. We then show that, in combination with *sim1a* knockdown, the midline shift of diencephalospinal axons is similar in *robo2; robo3*/+ and *robo2; robo3* mutant combinations. This genetic approach suggests that Robo3v2 blocks repulsion of Robo2. Finally, we show that overexpression of *robo3v2* but not *robo3v1* is sufficient to induce midline shifting of Robo2 dependent longitudinal axons. This finding demonstrates that Robo2 blocking activity is encoded within the first exon of the *robo3* locus, which is unique to *robo3v2*.
We propose that *sim1a* and *arnt2* negatively regulate the expression of *robo3v2* to ensure proper lateral positioning of longitudinal diencephalospinal axons by Robo2 mediated repulsion. Furthermore our findings reveal a link between the transcriptional programs that define neuronal subtype identity and the expression of receptors that guide aspects of their axonal projection paths.

INTRACELLULAR BINDING MOTIFS MEDIATE SALM1 TRAFFICKING IN HIPPOCAMPAL NEURONS

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The synaptic adhesion-like molecules (SALMs) are a class of cell adhesion molecules that contain an extracellular leucine-rich region (LRR), an immunoglobulin C2-like (IgC2) domain, a fibronectin type III (FNIII) domain, and a transmembrane domain. Of the five family members, only SALMs 1, 2, and 3 contain a cytoplasmic C-terminal PDZ-binding motif, which associates with the membrane-associated guanylate kinase (MAGUK) family of scaffolding proteins. The MAGUKs, including PSD-93, PSD-95, SAP97 and SAP102, are involved in clustering glutamate receptors at the synapse and regulate protein trafficking. SALM1 is unique among the SALMs, because deletion of its PDZ-binding motif (SALM1-delta PDZ) blocks its surface expression in heterologous cells. When expressed in hippocampal neurons, SALM1-delta PDZ decreases surface expression of SALM1 in the cell soma and dendrites, but not in the axons, suggesting that the PDZ-binding motif domain may influence cellular trafficking of SALMs to specific neuronal locations. Biochemistry and endoglycosidase H digestion assays indicate that SALM1-delta PDZ is retained in the ER in HeLa cells. However, when the entire C-terminal tail of SALM1 is deleted, SALM1 is detected on the cell surface. Using serial deletions, we have identified a region of SALM1 that contains a putative ER retention motif, which is not present in the other SALMs. Mutation of this region allows SALM1 to leave the ER and enhances its surface expression in heterologous cells. An increase in the number of protrusions at the dendrites and cell body was observed when this SALM1 mutant is expressed in hippocampal neurons. With electron microscopy, these protrusions appeared to be irregular, enlarged spines and filopodia. Future investigation of SALM trafficking may provide further insights into their regulation of neuronal cell adhesion and synapse formation.

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UNC-6/NETRIN MEDIATES DENDRITE SELF-AVOIDANCE IN *C. ELEGANS*

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Contact-dependent self-avoidance prevents overlap of sister dendrites and ensures non-redundant coverage of sensory fields. To study this phenomenon, we are using genetic methods and time-lapse imaging in *C. elegans* to examine a single type of nociceptive neuron with a complex dendritic architecture. PVD neurons (L + R) envelop the animal with a highly branched, orthogonal array of non-overlapping dendrites. The formation of these discrete sensory domains depends on the rapid withdrawal of sister dendrites upon contact with one another. Genetic ablation of UNC-6/Netrin perturbed PVD self-avoidance, resulting in overlapping dendritic branches. This finding raised the interesting question of how a diffusible cue like UNC-6/Netrin might control a contact-dependent mechanism. UNC-6/Netrin is secreted from ventral cells to create an apparent graded signal that steers axons along the dorsal/ventral axis. This UNC-6/Netrin gradient is not required for dendritic self-avoidance, however. Global expression of UNC-6/Netrin is sufficient to rescue the PVD dendritic overgrowth defect in an *unc-6* mutant and therefore confirms that UNC-6/Netrin is necessary for contact-mediated repulsion but acts independently of its source. This UNC-6/Netrin-dependent feature of PVD morphogenesis requires the cell-autonomous function of UNC-40/DCC and UNC-5. We propose that these receptors capture UNC-6/Netrin at the tips of outgrowing PVD branches and mediate the mutual withdrawal of apposing dendrites that contact this short range UNC-6/Netrin cue. In addition, mutants affecting other canonical UNC-6/Netrin signaling components (UNC-34/Ena, MIG-10/lamellopodia, MAX-2/P21 kinase) perturb PVD self-avoidance and therefore suggest that downstream mechanisms with known roles in axon guidance are also involved in dendritic morphogenesis. Our findings offer the first example of UNC-6/Netrin function in dendritic self-avoidance and provide a powerful model system in which to define the cellular and molecular mechanism of this effect.

ADAPTIVE PLASTICITY OF NEURONAL CIRCUITS DURING POSTNATAL MOUSE DEVELOPMENT

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Correct wiring of the central nervous system (CNS) during development enables the organism to respond to and interact with its environment. Attractive and repellent guidance cues, e.g. semaphorins, direct outgrowing neurons via the corresponding receptors, e.g. neuropilins, on the axonal surface. Previous studies revealed that absence of semaphorin-neuropilin signaling leads to characteristic miswiring of the spinal sensory-motor circuit. The defects comprise premature ingrowth of sensory and motor axons into the limbs, defasciculation, and altered dorsal-ventral choice of motor axons at the base of the limb. Surprisingly, some of these mutant mice are viable and thus amenable for postnatal analysis.
We therefore hypothesized that these embryonic axon wiring defects are corrected or compensated for during postnatal development.
To address this issue, we subject mouse mutants with abolished semaphorin-neuropilin signaling to a three-step approach: repetitive behavioral phenotyping using a wide-ranging test battery at three timepoints, detailed neuroanatomical analysis, and electrophysiological characterization of relevant neural circuits. Mutant animals showed significant impairments particularly in behavior tests aimed at monitoring motor coordination. Interestingly, mutants segregated into two clearly distinct groups of “wildtype-like performers” and “mutant performers”, and the later group improved in the execution of the behavioral tests over time. We are currently analyzing the anatomical and functional substrates for this observed improvement in locomotor capacities.
Our data suggests that lack of semaphorin-neuropilin signaling during the critical phase of neuronal wiring leads to specific deficits in motor coordination.

LOCAL UPREGULATION OF LIPRIN-A1 CAUSES DEPLETION OF PRESYNAPTIC BASSOON

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Communication between neurons occurs at specialized cell-cell contact sites called synapses, and the relative strength and weakness of these connections determines the contribution of a neuron within its neural network. The potency of individual postsynapses is modified for long periods of time by activity such that in the hippocampus, a synapse that is steadily activated can be potentiated even while its neighboring synapse, if stimulated to a lesser degree, can be depressed. It remains unknown, however, how the core components of the presynaptic active zone are altered in response to activity and whether or not presynapses are capable of functionally distinguishing themselves from their neighboring synapses. Here, we describe one particular active zone component, liprin-α1, which is degraded as a result of synaptic activity, and show that local upregulation of liprin-α1 causes a synapse-specific loss of the presynaptic scaffolding proteins bassoon and piccolo due to competitive binding to ELKS. These results uncover a new mechanism for locally regulating specificity and strength of synaptic connections and imply that presynapses may have a greater capacity for modulation than previously thought.

SONIC HEDGEHOG IN INTRARETINAL AXON GUIDANCE IN ZEBRAFISH

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Sonic Hedgehog (Shh) has been known for decades as a morphogen expressed by the embryonic vertebrate midline. More recently Hedgehog (HH) signaling has also been implicated in axon guidance of both commissural as well as retinal ganglion cell axons (Charron, et al., 2003; Kolpak, et al., 2005; Sánchez-Camacho and Bovolenta, 2008). Zebrafish mutant for *shh* or its receptor *smoothened* (*smo*) exhibit intraretinal axon guidance errors where RGC axons often fail to exit the eye through the optic nerve and instead become trapped within the eye and project posteriorly. How the HH pathway acts to guide RGC axons out of the eye is largely unknown. Here we set out to distinguish between eye patterning defects and direct axon guidance defects within the eye of HH pathway mutants in zebrafish. We used markers to control for normal eye patterning, a pharmacological approach to temporally control HH pathway activation, and cell transplant experiments to distinguish between cell-autonomous or non-cell-autonomous effects of HH pathway components for intraretinal axon guidance.

Both *shh* and *smo* mutants exhibit comparable intraretinal axon guidance errors, but only *smo* mutants lack optic stalk marker expression, such as *netrin1a* and Pax2, while *shh* mutants show normal optic stalk patterning. Our preliminary results show that intraretinal axon guidance errors can be induced with HH pathway inhibition late during eye development, after basic eye patterning has occurred. On the other hand, wash-off experiments indicate that early inhibition of HH signaling is sufficient to induce intraretinal axon guidance errors. Thus, it may be that the HH signaling pathway has a role in intraretinal axon guidance both during early and late eye development. Using cell transplants, we found that Shh and,surprisingly, Smo act non-cell-autonomously in intraretinal axon guidance. Experiments are underway to test whether the non-autonomous role of Smo is due to fasciculation between axons.

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AXON EXTENSION OCCURS INDEPENDENTLY OF CENTROSOMAL MICROTUBULE NUCLEATION

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The centrosome is the classical site of microtubule nucleation and is thought to be essential for axon growth and neuronal differentiation; processes that require microtubule assembly. Here, we found that the centrosome loses its function as a microtubule organizing center (MTOC) during neuronal development. Axons still extended and regenerated through acentrosomal microtubule nucleation and axons continued to grow after laser ablation of the centrosome in early neuronal development. Thus, decentralized microtubule assembly enables axon extension and regeneration and, after axon initiation, acentrosomal microtubule nucleation arranges the cytoskeleton, which is the source of the sophisticated morphology of neurons.

VISUALIZATION OF NEURONAL INTEGRIN SIGNALING USING BIMOLECULAR FLUORESCENCE COMPLEMENTATION

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Axons navigate over long distances to innervate their targets and establish synaptic connections during neuronal development. During this process, growth cones receive and integrate both attractive/permissive and repulsive cues to steer axons to correct targets. Although much is known about downstream components of these guidance cue signaling pathways, it remains to be elucidated how these signaling pathways are integrated within extending neuronal processes. For example, it is possible that repulsive guidance cue signaling requires the concomitant modulation of permissive/attractive signaling. In neurons, integrin signaling can act in a permissive fashion to mediate cell attachment, neurite outgrowth, and axon pathfinding through interactions with the extracellular matrix (ECM). Recent work on the semaphorin signaling cascade suggests that plexin receptor activation by semaphorins acts to negatively regulate integrin function. To gain a better understanding of the spatial and temporal activation of integrin signaling in live neurons, and to determine how other guidance signaling pathways might modulate integrin signaling during axon guidance, we have developed a Bimolecular Fluorescence Complementation (BiFC)-based reporters to directly visualize integrin signaling events. We have constructed BiFC probes to allow visualization *in vitro* and *in vivo* of the hierarchical activation of FAK and p130Cas, two downstream components of integrin signaling. *In vitro* re-plating assays and pharmacological manipulation of integrin function show that these BiFC probes serve as reliable integrin signaling sensors in living cells. By utilizing BiFC live imaging and neuronal culture techniques, we are currently investigating the relationship between semaphorin and integrin signaling pathways in regulating axon pathfinding. Our initial results suggest that the cell morphological changes induced by acute treatment with secreted semaphorins correlate with a reduction of BiFC intensity using a FAK/Src BiFC pair. We have also begun characterizing our BiFC sensors *in vivo* in chicken and mouse using different axon guidance paradigms. This approach should allow us to visualize the temporal and spatial regulation of integrin signaling during axon pathfinding and target recognition, and will also address how repulsive signaling modulates integrin activity during the neuronal process guidance.

ROLES OF PSD-95 AND CAMKII IN THE STABILIZATION OF AXODENDRITIC CONTACTS

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Changes in connectivity are thought to underlie many forms of neuronal plasticity, but little is known about the molecular mechanisms that stabilize transient synaptic connections. Previous work has shown that CaMKII can increase the strength of existing connections (Pratt et al., 2003) and that overexpression of activated CaMKII in post-synaptic neurons selectively promotes new contact formation (Pratt et al., 2008). Here we sought to examine the dynamics of PSD-95 and CaMKII at axodendritic contacts (AD), and to correlate their presence and phosphorylation state with the formation, loss or stability of the AD contact.

We performed time-lapse imaging on pairs of cultured rat visual cortical neurons sparsely transfected with dsred-PSD95 and GFP-CaMKII (postsynaptic), or CFP (presynaptic). We found neuronal pairs where a CFP axon contacted one or more PSD95 and CaMKII-labeled dendrites and imaged these connections every 20 minutes for 3 hours. The dynamics of PSD95 and CaMKII puncta at transient or stable AD contacts were used to determine rates of gain and loss for both the puncta and AD contacts. Cultures were stained post-hoc against phospho-T286 to look at constitutive activation of CaMKII.

Rates of gain and loss of AD contacts were well-balanced, consistent with previous data (Pratt et al. 2008). PSD-95 and CaMKII puncta at AD contacts were dynamic and turned over faster than the AD contacts. PSD-95 and CaMKII puncta were more likely to be present at stable contacts at some point during the experiment, with PSD95 puncta spending significantly more time at stable AD sites. Next we examined if there was a correlation between the activation state of CaMKII and the turnover of AD contacts and/or PSD95 and CaMKII puncta. We found a significant increase in accumulation of activated CaMKII at stable AD contacts as opposed to transient ones. These data suggest that both activation of CaMKII and the presence of PSD-95 may confer stability upon AD contacts.

We then performed time-lapse imaging on AD contacts between neurons loaded with FM4-64 dye (presynaptic) and pyramidal neurons transfected with GluR2-YFP (postsynaptic) in order to determine how PSD-95 overexpression can alter the stability of AD contacts. Rates of gain and loss for GluR2-YFP puncta were well-balanced. When PSD95-CFP was co-transfected with GluR2-YFP, rates of gain and loss remained balanced but were lower than GluR2-YFP alone. Further, overexpression of PSD95 promoted an increase in the percentage of stable contacts as well as a significant increase in the average contact lifetime. Taken together, this indicates that PSD95 overexpression stabilizes GluR2-containing axodendritic contacts.

$GABA_B$ RECEPTOR SIGNALING DURING AXON GUIDANCE IN THE ZEBRAFISH

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During development, growth cones encounter a multitude of extracellular cues. The proper integration of these signals determines the direction of axon migration. Despite the absence of functional neural circuitry, neurotransmitters are expressed throughout the developing brain. While there is evidence that neurotransmitters affect neurite outgrowth and growth cone turning *in vitro*, there is little evidence to support a role for neurotransmitters in axon guidance *in vivo*. Our lab has previously shown that the excitatory neurotransmitter glutamate, acting through the metabotropic glutamate receptor (mGluR1), reduces growth cone sensitivity to multiple repellent cues *in vitro*. We find that the activation of the metabotropic GABA receptor ($GABA_B$) can similarly reduce axon responsiveness to repellents. The selective $GABA_B$ agonist baclofen decreases chick retinal ganglion cell (RGC) sensitivity to the repellent Slit2. To test whether signaling through the $GABA_B$ receptor is necessary for proper axon guidance *in vivo* we have generated an mRFP-tagged dominant negative $GABA_B$ receptor (R1C). When expressed in HEK293 cells, R1C blocks the surface expression of the ligand binding subunit of the $GABA_B$ heterodimer. Expression of this construct in chick RGC's blocks baclofen's effect on Slit2 induced collapse. These data demonstrate that R1C functionally blocks $GABA_B$ signaling. We are using the GAL4/UAS system to restrict R1C expression to select populations of neurons in the embryonic zebrafish. GABA immunoreactivity is detected anterior and posterior to the optic chiasm at the time when retinal axons first reach the midline (36 hours post fertilization) putting it at the right place and time to influence retinal axon guidance. UAS:R1C transgenic fish were crossed to an Ath5:GAL4 line to restrict R1C expression to RGCs. Preliminary analysis indicates that there is a reduced number of RGCs and a thin retinal projection in 70% of Ath5;R1C embryos 48 hours post fertilization (hpf) compared to 16% in Ath5;Citrine controls. In pharmacological studies, 30% of embryos treated with the $GABA_B$ antagonist CGP54626 from 33hpf to 48hpf displayed thin retinal projections compared to 0% of vehicle treated controls. These data are consistent the R1C data. In 30% of Ath5;R1C embryos a small subset of retinal axons wander anteriorly at the chiasm. On-going work is aimed at determining whether $GABA_B$ is required for RGC survival and whether retinal guidance defects are secondary to the loss of RGCs.

THE ORIGIN AND DEVELOPMENT OF CHANDELIER CELLS IN MOUSE NEOCORTEX

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In mammalian neocortex, diverse GABAergic interneurons display distinct physiological properties and connectivity patterns, and regulate the balance and dynamics of cortical network. Chandelier cells (CHCs) are one of the most distinct classes of interneurons, which exclusively innervate pyramidal cell axon initial segments (AISs), the sites of action potential initiation. CHCs were nicknamed “veto cells” based on their potential ability to silence populations of pyramidal neurons in cortical network. However, recent studies suggest that CHC→pyramidal cell transmission may in fact be excitatory under certain conditions and thus may represent the most powerful trigger of action potentials. Whether inhibitory or excitatory, CHCs are likely crucial regulators of pyramidal neuron spiking and synchrony. Reduced synaptic marker expression in CHCs in human prefrontal cortex is a highly reproducible molecular pathology in schizophrenia.

Although discovered more than 3 decades ago, the development and function of CHCs have remained elusive due to lack of reliable methods. Through genetic fate mapping of precursors in the subventricular zone (SVZ) using Nkx2.1-CreER knockin mice, surprisingly, we discovered that CHCs continue to be generated at and after E17, when the medial ganglionic eminence, which gives rise to majority of cortical GABA neurons, has already disappeared. This result suggested the ventral ridge of the SVZ, which remains Nkx2.1+, as a source of late-born CHCs. Young CHCs appeared to migrate along the ventricular wall and then spread mediolaterally along the cortical ventricular zone; they entered cortical plate through radial migration, reaching the superficial layers by P3. By P7, the superficial layer2 was densely packed with young CHCs throughout the cortex. Strikingly, at P14 there was a marked reduction in the number of CHCs; this reduction became even more substantial at P21. By P21, CHCs were well separated with each other and displayed chandelier-like axon arbors with dense arrays of synaptic cartridges along AIS of pyramidal neurons. Patch-clamp recording demonstrated that these CHCs are fast-spiking and electrically coupled between nearby CHCs. Our results suggest massive cell death as well as substantial axon pruning during the maturation of CHCs; they imply fierce competition among CHCs during the establishment of their innervation territory to pyramidal cells at a stage when cortical circuit formation is strongly influenced by neural activity and experience. The reliable genetic access to CHCs sets the stage for a comprehensive analysis from cell birth to their integration and function in cortical circuits.

SEMA3A AND L1CAM FAMILY, A MOLECULAR CODE FOR CELL TYPE RECOGNITION BY GABAERGIC INTERNEURON

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GABAergic interneurons are fundamental component in neural processing and their specific innervation patterns are though to be the building block for physiological brain function and computing. However the molecular and cellular mechanisms that assemble inhibitory local circuits remain largely unknown. In cerebellar cortex, molecular layer GABAergic interneurons are key regulators of cerebellar signal coding and memory formation by sending specifically their axons to innervate the Purkinje cells. Here, we show that a combination of both secreted axon guidance and recognition molecules of L1CAM family is sufficient to trigger target cell recognition by molecular layer GABAergic interneuron's in vivo. Using BAC transgenic reporter mice for cell-type specific gene-expression profiling of secreted SEMAPHORIN molecules, we identified that SEMAPHORIN3A (SEMA3A) expression picked precisely at relevant time-point of GABAergic local circuit formation. In vitro, in a co-culture model, we found that semaphorin3A (SEMA3A) secreted by CHO cells attracts GABAergic interneurons axons and triggers their local specific branching. In vivo, the injection of these heterologous cells expressing SEMA3A ectopically in the granule cell layer is able to disrupt the "crystal" like organization of molecular GABAergic interneurons and attracts their axons in this ectopic territory. Moreover we found that both in vitro and in vivo, the co-expression of SEMA3A and the L1CAM family recognition molécules, Neurofascin, but not their respective expression alone, are able to induced heterologous cells innervation by molecular GABAergic interneurons. These results suggest that specific combination between axon guidance molecules and L1CAM family is sufficient to specify cell type recognition in a space and timely dependent manner.

A CUE FOR VISION: HOW EPHB1, EPHB2, EPHRIN-B1, AND EPHRIN-B2 MEDIATE RETINOCOLLICULAR MAPPING

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RGC axon mapping to the superior colliculus (SC) is critical for integrating both the visual field and head orientation to mediate appropriate eye movement. Previously, the Henkemeyer laboratory collaborated with other laboratories using *EphB2*$^{-/-}$*; EphB3*$^{-/-}$ (receptor null mutant) and *EphB2*$^{LZ/LZ}$*; EphB3*$^{-/-}$ (EphB2 intracellular signaling deficient mutant) mice to establish that EphB2 forward signaling mediates ventral RGC axon attraction and termination to the medial SC. However, the EphB2 intracellular component essential for retinocollicular mapping is unknown. Also, EphB1, ephrin-B1, and ephrin-B2 are expressed in the visual system, but their role in mapping is unknown. Thus, we carried out a comprehensive examination of EphB and ephrin-B mutant mice using DiI to trace dorsal and ventral RGC axons to determine how these molecules affect mediolateral retinocollicular mapping. Using EphB2 receptor kinase and PDZ binding motif point mutants, we determined that the EphB2 tyrosine kinase domain, not PDZ binding motif, is critical for ventral RGC axon retinocollicular mapping. Additionally, ventral, not dorsal, RGC axons require EphB1 for axon termination in the SC. Furthermore, ephrin-B2, not ephrin-B1, mediates dorsal RGC retinocollicular mapping, whereas both mediate ventral RGC axon retinocollicular mapping.

NETRIN, FRAZZLED AND UNC-5 ARE REQUIRED FOR LAYER-SPECIFIC TARGETING OF PHOTORECEPTOR AXONS IN *DROSOPHILA*

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The visual system of vertebrates and invertebrates consists of a variety of neuron subtypes, whose arborizations are frequently organized into distinct columns and layers. Organization of neuronal connections into layered functional pathways enables parallel processing of several visual features within a network, such as motion and color detection.
We use the *Drosophila* visual system as a model to investigate the mechanisms that regulate layer-specific axon targeting. Photoreceptor neurons (R-cells, R1-R8) extend axons into the optic lobe. R1-R6 axons target to the lamina, while R8 and R7 axons terminate in two distinct layers in the medulla, called M3 and M6.
We show that the secreted Netrin ligands, the attractive Frazzled and the repellent Unc-5 guidance receptors play a central role in regulating layer-specific axon targeting of R8 axons to the M3 layer by providing positional information. Loss of both *Netrin A and B* results in similar R8 axon targeting defects as loss of its receptors *frazzled* and *unc-5* in R-cells. Netrin B is expressed by lamina neurons L3, as well as target neuron subtypes and is localized in the medulla layer M3. Frazzled is expressed in R8, as well as target neuron subtypes. We propose that Frazzled has a dual function in the developing visual system: (1) layer-specific localization of Netrin B through target neurons and (2) guidance of R8 axons to their specific target layer M3. Unc-5 shows a highly dynamic layer-specific expression suggesting a role in organizing layer-specific targeting of R8 axons and target neuron arborizations.

ACTIVITY-DEPENDENT RETROGRADE LAMININ A SIGNALS TRIGGER THE PRESYNAPTIC INTEGRIN/FAK/NF1/CAMP PATHWAY TO CONFINE SYNAPTIC GROWTH

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Activity-induced synaptic retrograde signals predominantly potentiate presynaptic properties. In this study, we describe a presynaptic integrin signaling pathway to confine experience-dependent synaptic growth at *Drosophila* neuromuscular junctions. This non-conventional integrin signaling requires FAK-NF1 complex formation to induce high levels of cAMP for confining synaptic growth. The ligand triggering presynaptic integrin activation is postsynaptically muscle-derived Laminin A (LanA) that presents at synaptic clefts and locally induces apposing presynaptic integrin signaling. The level of LanA at NMJs is regulated by synaptic activities and anterograde Wnt/Wg signaling, both modulating NMJ growth in LanA-dependent manner. Indeed, LanA downregulation precedes structural expansion in the experience-dependent NMJ growth paradigm. In conclusion, at baseline neural activities, synaptic cleft-localized retrograde LanA signals locally trigger the presynaptic integrin/FAK/NF1/cAMP pathway to confine synaptic growth. Hyper activities downregulate LanA levels at synaptic clefts, thus relieving synaptic growth inhibition.

THE EFFECT OF β-AMYLOID ON THE GROWTH AND RETRACTION OF NEURITES IN CULTURED HIPPOCAMPAL NEURONS

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Beta-amyloid (Aβ) is a major component of the amyloid plaques, which are a hallmark of Alzheimer's Disease (AD) together with neurofibrillary tangles. A number of recent evidence points to synaptic loss and alterations in neurites morphology during the early development of AD (Spires 2005). To date, Aβ has been shown to increase the actin polymerization at the neurite tips (Mendoza-Narajno 2007, Henriques 2010), and to inhibit neurite outgrowth in developing neurons (Petratos 2008).

We are addressing the question of the possible effect of Aβ on the development of neurites using various microscopy techniques. By time-lapse video microscopy, we were able to follow the response to exogenous Aβ and to study the reversibility of its effect on the cultured neurons at early days in culture. Moreover, the effect of Aβ on the morphology of actin cytoskeletal structure was studied by immunofluorescence with confocal and Stimulated Emission Deletion (STED) microscopy.

We noticed that there are diverse effects of Aβ on dendrites and axons, in terms of neurite length and re-growth, possibly due to different membrane compositions. In addition, we show that the exogenous application of Aβ leads to the formation of abnormal F-actin aggregates in the growth cones visualized by STED microscopy. We are currently analyzing other proteins that may participate in the formation of, or be trapped in these actin structures.

Aβ has been found to interact with α-synuclein, enhancing their properties to form aggregates. They are both present in Lewy bodies, which are proteinaceaous inclusions characteristic of neurodegenerative diseases. Therefore we investigated the possible enhancement or rescue of the Aβ's effect by α-synuclein, by comparing the phenotype of wild-type mice neurons against that of neurons from a mice strain carrying a deletion of α–synuclein gene, as well as that of neurons reconstituted by electroporation.

THE WND/DLK MAPKKK AND DOWNSTREAM SIGNALING CASCADE REGULATE APP TRANSPORT IN *DROSOPHILA* AXONS

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An important problem for neurons is to transport molecules and organelles over long distances in axons. Microtubule-based kinesin and dynein motor proteins play essential roles in neuronal function and survival. Important questions involve regulation: how do different cargos bind to or detach from motors, and how are these interactions regulated? In *Drosophila* we have found that a conserved signaling pathway that regulates nerve terminal structure plays a role in axonal transport. A key component of this pathway is a MAP Kinase Kinase Kinase named Wallenda (Wnd) in *Drosophila*, and DLK in vertebrates. Previous studies suggested that Wnd and downstream JNK signaling regulate the binding of a JNK scaffold protein JIP1 (also known as APLIP1 in *Drosophila*), with the Kinesin-1 motor [(1)]. Here we test the hypothesis that by regulating the binding of JIP1 to Kinesin-1, Wnd/JNK signaling regulates the transport of certain Kinesin-1 cargo *in vivo*. A candidate cargo is the Alzheimer's Disease associated protein, APP, which is transported in axons and interacts biochemically with JIP1, both in vertebrates and *Drosophila* [(2)].When Wnd or JIP1 function is disrupted through loss-of-function mutations, APP-YFP vesicles still enter axons, however they display an increased retrograde flux and pausing frequency in *wnd* loss-of-function mutants, and an increased number of stationary particles in *jip1* null mutants. Moreover, a greater proportion of the vesicles move with a slower anterograde velocity, suggesting that a different motor other than Kinesin-1 may be responsible for their mobilization in axons when Wnd or JIP1 is absent. Importantly, transport another class of cargo, ANF-GFP labeled dense core vesicles, is not affected in *wnd* mutants, supporting the model that Wnd regulates the transport of some but not all cargo in axons. Finally, synaptic overgrowth caused by over-expression of APPL [(3)], is suppressed by mutations in *wnd* and *jip1*. This suggests that the class of vesicles regulated by Wnd may play a role in synaptic development.

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NGR1 IN RECOVERY FROM CHRONIC SPINAL CORD INJURY

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A common opinion is that blockade of axonal growth inhibitors can be beneficial only in a time frame close to the trauma, although recent work suggests that growth can be promoted long after injury by a complex combination of cellular, surgical and molecular interventions. Because axonal tracts remain intact in regions proximal to the site of spinal cord contusion and remain exposed to myelin and myelin debris, we reasoned that blockade of myelin inhibitors long after SCI might stimulate axon growth and recovery.

As a first test, we utilized a conditionally targeted (Flox'ed) NgR1 allele, which is expressed at WT levels prior to Cre exposure. In mice with a ß-actin promotor cre/Esr1 transgene, tamoxifen treatment leads to highly efficient NgR1 rearrangement and loss of protein within a week. We subjected mice to dorsal hemisection injury and then waited for 75 days, a time when natural recovery is complete. All mice were treated with tamoxifen at this stage, and for those with Cre/Esr1, NgR1 expression was lost. Over the ensuing 75 days the mice without NgR1 expression improved open field walking scores. CST axonal tracing demonstrated sprouting into the injury site.

We extended the chronic SCI experiment to include pharmacological intervention. A cohort of 64 rats survived thoracic spinal cord contusion injuries, and exhibited stable motor scores without fluctuation between 6-12 weeks after injury. The average BBB score at 12 weeks was 7.75 + 0.12, meaning that the majority of rats were capable of hindlimb movement, but not weight support. After intracerebroventricular (i.c.v.) catheter placement, the animals were randomized to receive either NgR(310)ecto-Fc or control IgG protein for 12 weeks at a dose of 0.29 mg/kg/d. There was a significant improvement over three months in the NgR(310)ecto-Fc group, but not the control group and the improvement of each animal's BBB score in the NgR(310)ecto-Fc treated group was significantly greater than in the control group. Because most control rats did not step or support weight with the hindlimbs, detailed gait analysis was not possible. The most conspicuous behavioral change was the conversion from hindlimb non-weight-bearing to hindlimb weight-bearing locomotion. Ten of the NgR(310)ecto-Fc treated animals converted to weight bearing, while only one control rat did so over this three month period. Increased serotonin (5HT) fiber length was observed in the lumbar spinal cord here after treatment of chronic SCI. NgR(310)ecto-Fc treatment of chronic spinal contusion improves neurological recovery.

SECRETED SEMAPHORIN CONTROL OF DENDRITE AND SPINE MORPHOLOGY DURING DEVELOPMENT AND IN THE ADULT MOUSE CNS

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The wiring of neuronal circuits requires the precise formation and refinement of synapses during development. The majority of excitatory synapses in the mammalian central nervous system (CNS) are formed on dendritic spines. Spine morphology, density and distribution are critical for proper synaptic transmission. We showed that certain secreted semaphorins are critical for the elaboration of dendritic spine morphology and synapse structure in the mouse CNS (*Tran et al., 2009*). Mice deficient in Sema3F signaling, including mice lacking its holoreceptor components neuropilin-2 (Npn-2) and plexin A3 (PlexA3), exhibit dramatically altered spine morphology, spine distribution, and synaptic ultrastructure in hippocampal and cortical neurons. Both hippocampal and cortical neurons from Npn-2 null mice exhibit altered synaptic transmission, consistent with our *in vitro* and *in vivo* observations. In contrast, the closely related protein Sema3A, and its Npn-1/PlexA4 holoreceptor components, promote the elaboration of cortical basal dendritic arbors but do not influence spine morphogenesis. These distinct secreted semaphorin functions are likely governed by restricted localization of Npn-2 to select dendritic domains of cortical pyramidal neurons. We are using both *in vitro* and *in vivo* approaches to investigate the intracellular signaling mechanisms utilized by secreted semaphorins to regulate dendritic growth and branching, and spine morphology, and synaptogenesis. Chimeric Npn-1^{ecto}/Npn-2^{cyto}, Npn-2^{ecto}/Npn-1^{cyto} receptors, in combination with PlexA3^{ecto}/PlexA4^{cyto} and PlexA4^{ecto}/PlexA3^{cyto} chimeric receptors, allow for *in vitro* and *in vivo* experiments focused on how selective localization of these receptor complexes is achieved. In addition, these chimeric receptors also assist in determining how secreted semaphorins differentially regulate dendritic outgrowth and spine morphology. Our findings suggest that tight spatial regulation of guidance cue responses contribute to the generation of complex connectivity patterns in the CNS, and underscore the necessity of understanding the mechanisms that serve to selectively localize guidance cue receptors to dendritic regions where they influence spine morphology and synaptogenesis.

LTP AT EXCITATORY SYNAPSES IS MODULATED BY SPECIFIC INHIBITORY CIRCUITS AND BY VISUAL EXPERIENCE.

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Layer 4 (L4) is the main input layer in primary cortex. It receives direct thalamocortical inputs and sends major projections to L2/3. Although L4 architecture renders it an important gate for cortical activation by visual stimuli, the plasticity of L4 synapses is poorly understood. One of the main differences between L4 and other cortical layers is that to date L4 excitatory synapses have shown mono-directional plasticity (only depression), and this depression can be induced only before the classical critical period for visual cortex begins. Here we show that, contrary to what is expected, L4 excitatory synapses have the ability to undergo long term potentiation (LTP) in response to a frequency-dependent learning rule (144.17 ± 16.35% of baseline after induction; $p = 0.014$; $n = 20$). The LTP is developmentally regulated and is inducible specifically during the critical period for visual cortical plasticity. Furthermore, the LTP in L4 is occluded by monocular deprivation (MD; 88.21 ± 7.33% of baseline; two way ANOVA: $p < 0.01$; Control: $n = 9$; Deprived: $n = 10$). We tested the possibility that inhibitory circuits in L4 may control the induction of this novel form of LTP and found that MD in the presence of intracortical blockade of $GABA_A$ receptors restored LTP induction (116.65 ±3.16% of baseline; two way ANOVA: $p = 0.27$; Control: $n = 7$; Deprived: $n = 9$). We found that the MD-dependent impairment of LTP induction was mimicked by chronic intracortical infusion of Diazepam (81.7 ± 8.4% of baseline; two way ANOVA: $p < 0.01$; Control: $n = 7$; Deprived: $n = 8$), but not Muscimol (111.98 ± 7.67% of baseline; two way ANOVA: $p > 0.05$; Control: $n = 7$; Deprived: $n = 9$). However, both diazepam and muscimol reduced the spatio-temporal pattern of cortical circuit activation measured with voltage sensitive dye imaging. Together our results suggest that the interplay between benzodiazepine sensitive inhibitory circuits and recurrent excitatory synapses in L4 are critical for postnatal cortical circuit refinement and for the development of visual function.

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A FORWARD GENETIC SCREEN FOR GENES REGULATING CONNECTIVITY IN THE MOUSE CENTRAL NERVOUS SYSTEM

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Proper functioning of the mammalian CNS requires the formation of precise neuronal circuits. Reverse, and to a lesser extent forward, genetic screening strategies have successfully identified genes required for brain development. We have implemented an ENU-based forward genetic screen based on neurofilament immunohistochemistry of postembryonic mouse brains, and we have identified several heritable mutants affecting brain development with phenotypes ranging from specific axon guidance defects to more general structural alterations. For example, line #4182 displays defects in projection of the anterior commissure (AC) that affect both AC branches. In this line, the posterior branch of AC is reduced to a small number of defasciculated fibers that fail to cross the midline. Severe defasciculation of the fasciculus retroflexus is also observed. A different line (#4783) displays defects in corpus callosum (CC) formation. Although CC fibers do form normally in the lateral brain regions, the CC fails to cross the midline and forms prominent Probst bundles. Line #3005 displays a defasciculated CC and hydrocephalus. We have mapped this mutant to the DLG5 locus, which encodes a member of the MAGUK (membrane associated guanylate kinase) family of proteins and has previously been shown to be involved in epithelial cell-polarity determination. Given structural similarities between Dlg5 and other MAGUK proteins with established functions in synapse formation, and also the ability of Dlg5 to associate with proteins known to regulate synaptogenesis, we have investigated how Dlg5 functions in neural development. We find that Dlg5 is required for dendritic spine formation in cortical neurons. The mutation in DLG5 we have isolated in our screen is a missense mutation within the Dlg5 SH3 domain. SH3 domains typically mediate assembly of protein complexes, suggesting that an unidentified Dlg5-interacting protein is critical for dendritic spine formation. Current work on how Dlg5 regulates spine formation and synaptogenesis will be presented. Ultimately, our unbiased forward genetic approach has allowed us to identify genes required for establishment of proper neuronal connectivity, providing further understanding of mechanisms that regulate multiple aspects of brain development.

HUMAN DSCAMS ARE FUNCTIONALLY CONSERVED WITH *DROSOPHILA* DSCAM[TM1]

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Drosophila Dscam gene encodes thousands of isoforms that share two alternative transmembrane/juxtamembrane (TM) domains. Dscam[TM1] isoforms are localized in the dendrites and control dendritic elaboration, while Dscam[TM2] isoforms are localized in the axons and control axonal arborization. However, vertebrates only have two DSCAM molecules, DSCAM and DSCAM-Like 1 (DSCAML1), which are encoded by two orthological genes. Using ClustalW and MEGA, we have conducted a phylogenetic analysis for the TM domains of Dscam/DSCAMs. Accordingly, they can be divided into four major categories, insect Dscam[TM1], insect Dscam[TM2], vertebrate DSCAM, and vertebrate DSCAML1 groups. In order to test the potential functional conservation between invertebrate Dscams and vertebrate DSCAMs, a series of cross-species assays were performed to examine the subcellular localization and function of human DSCAM and DSCAML1 in the *Drosophila* brain. We found that both human DSCAM and DSCAML1 are strictly targeted to dendrites of *Drosophila* neurons and can partially but substantially rescue the larval lethality of *Drosophila* Dscam mutants. Synergic rescuing effect was observed when *Drosophila* Dscam[TM1] isoform was combined with Dscam[TM2] isoform, but not occurred when two Dscam isoforms with the same TM domain were combined. Similarly, synergic rescuing effects were also detected when human DSCAM and DSCAML1 were combined with Dscam[TM2] isoforms, but not occurred when they were combined with Dscam[TM1] isoforms. Taken together, we conclude that Human DSCAMs are functionally conserved with *Drosophila* Dscam[TM1]. Consistently, we discovered that human DSCAM and DSCAML1 rescue the dendritic self-avoidance defects of *Drosophila* dendritic arborization (da) neurons, but not rescue the axonal bifurcation defects of *Drosophila* mushroom body neurons.

A NOVEL CONSERVED ELONGIN BC-BOX PROTEIN REGULATES THE SLIT/ROBO PATHWAY IN AXON GUIDANCE

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The ventral axon guidance of AVM mechanosensory neurons in C. elegans requires the cooperative activity between the repulsive *slt-1/sax-3* (Slit/Robo) pathway and the attractive *unc-6/unc-40* (Netrin/DCC) pathway. Here we report that EBAX-1 (Elongin BC-Binding Axon regulator), a newly identified protein bearing conserved Elongin BC- and Cullin 2-interacting motifs (BC-box and Cul2-box), regulates AVM axon guidance through the *slt-1/sax-3* pathway. EBAX-1 is expressed widely in mid-late embryos and highly enriched in the embryonic nervous system. Functionally tagged EBAX-1 shows cytoplasmic and axonal localization in neurons. We find that during development *ebax-1* functions in the same pathway as slt-1/sax-3 and in parallel with *unc-6/unc-40* to guide AVM axon ventral growth cell-autonomously. Biochemical studies show that EBAX-1 and its mammalian homologs bind Elongin-BC through the BC-box. The BC-box and Cul2-box are essential for EBAX-1 to function in AVM axon guidance. Previous studies have demonstrated that such BC-box proteins often acts as a substrate recognition subunit in the multiple-subunit E3 ubiquitin ligase complex containing Elongin B, Elongin C, Cullin 2 (Cul2) and Ring-box protein 1 (Rbx1). We will present our analyses how EBAX-1 regulates axon guidance through the E3 ligase complex.

RECONSTITUTION OF TOPOGRAPHIC GUIDANCE IN THE PRESENCE OF GROWTH CONE ADAPTATION

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The chick retinotectal projection is a classical model for investigating the development of topographic projections, axonal mappings that preserve neighborhood relationships. A multitude of mechanisms contributing to map formation have been elucidated, but their integration into a comprehensive model has remained controversial. By this project, we try to build up a novel, parsimonious evidence-based model through successive improvements of computational simulation combined with step-by-step experimental validation. Previously, we have shown that counter-graded ephrin / Eph forward and reverse signals, when antagonistically integrated, are sufficient to explain topographic targeting of chick primary retinal growth cones (GCs). In vitro we corroborated this notion by the first-time reconstitution of proper topographic behavior of retinal GCs in binary choice assays using recombinant guidance cues.
Here, we upgrade our model by implementing experimental evidence on GC adaptation, which conceptually is difficult to reconcile with topographic targeting. We have, however, devised an adaptation strategy that can explain both, continuous adaptation and topographic precision. While substantial experimental evidence for adaptation towards ephrin-A forward signaling has been gathered, we are currently attempting to validate the model predictions regarding adaptation of reverse signaling by manufacturing appropriate EphA receptor substrates for adaptation assays. Furthermore, we extend our previous results by establishing a new method for fabrication of functional countergradients of ligand and receptor on two tightly arranged glass surfaces with defined spatial properties. Each glass surface carries one of the proteins in a graded pattern created with micro-contact or diffusive printing. Retinal axons are supposed to grow into the µm-range gap between the glass surfaces and get access two both proteins. The spatial separation of EphA3 and ephrin-A5 prohibits an interaction and masking of the surface bound proteins, which would be expected if both proteins were arranged on the same surface. These novel “sandwich countergradients“ will allow to design in vitro assays with conditions close to the in vivo distribution of EphA3 and ephrin-A5 known from quantitative histochemistry in embryonic tissue.
By this strategy of iterative model refinement and experimental validation, we hope to arrive at an understanding of topographic mapping that is at the same time accurate with respect to experimental detail and theoretically consistent.

CST AXONS THAT REGENERATE AS A RESULT OF PTEN DELETION FORM SYNAPSES CAUDAL TO A SPINAL CORD LESION

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Previously Park *et al.* (Science 322, 963-966 (2008)) have demonstrated that mice with PTEN (phosphatase and tensin homolog) deleted in retinal ganglion cells exhibit robust axonal regeneration after optic nerve injury. In more recent studies, our group has demonstrated that genetic deletion of PTEN in the sensorimotor cortex enables CST axons to regenerate past even complete crush injuries of the spinal cord. Here, we assess whether corticospinal tract (CST) axons that have regenerated following spinal cord injury (SCI) form synapses in caudal segments. Mice homozygous for the floxed PTEN gene ($PTEN^{f/f}$) were injected with Cre-expressing adeno-associated virus (AAV-Cre) at P1 to delete PTEN from the right sensorimotor cortex. At 2 months of age, crush injuries were produced at thoracic level 8 (T8), mice were allowed to survive for 10 weeks, and then biotinylated dextran amine (BDA) was injected into the right sensorimotor cortex to trace the descending CST, and mice were allowed to survive for 2 weeks post-injection. Spinal cord segments that included the lesion were sectioned in the sagittal plane on a Vibratome®, stained for BDA, embedded in Spurr's resin and sandwiched between layers of Aclar film for visualization under light microscopy. BDA-labeled axonal swellings suggestive of synaptic boutons were identified under light microscopy, and then the block was prepared for electron microscopy. Of 13 bouton-like structures examined so far by electron microscopy, 3 are definitive synapses based on the presence of a contact zone and post-synaptic density. These results indicate that axons from neurons with PTEN deleted can regenerate past and form synapses caudal to a spinal cord lesion, indicating that manipulation of the PTEN pathway can produce a robust and potentially meaningful regenerative response.

DEVELOPMENT OF RNAI VECTORS ELICITING CELL TYPE-SPECIFIC, TRACEABLE GENE KNOCK DOWN IN THE NEURAL TUBE

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The pathfinding behaviour of commissural axons of the developing neural tube is one of the best studied model systems in axon guidance. Typically, the commissural axons express receptors or cell adhesion molecules that detect cues emanating from their intermediate target, the floor plate. To allow us to test the molecular and cellular interactions of novel axon guidance candidates, we report the design and synthesis of cell type-specific RNA interference constructs for use in the chick neural tube. The vectors are based on artificial derivatives of pri-miR-30 (Das et al, 2006). Cell type-specific promoters/enhancers drive the expression of a fluorescent protein marker, followed by the miR30-RNAi transcript (located within the 3'-UTR of the fluorescent protein). When electroporated into the developing neural tube, these vectors elicit cell type-specific, traceable gene knock down. Two genes are able to be targeted from a single RNAi vector, or they can be mixed prior to electroporation, to enable the simultaneous knock down of two or more genes in independent regions of the spinal cord (ie floor plate and commissural neurons). We have applied the new vectors to knock down several genes (Axonin1/TAG-1, Shh, Wnt7a, Glypican1) in proof-of-principle experiments. Not only do the vectors elicit dramatic knock down of gene expression (detectable by in situ hybridization and immunohistochemistry), but the level of knock down is sufficient to reproduce the expected guidance defects upon perturbation of genes with known axon guidance functions. All vectors express bright fluorescence to enable direct tracing of the cells experiencing knock down. In addition, we describe an in vitro method to rapidly assess the relative efficiency of new miRNAs against their target gene. The advancements of the in ovo RNAi technique that we describe will markedly enhance the biological validity, experimental precision and combinatorial possibilities of studies in RNAi, axon guidance and neural development.

ARFGAP1 IS REQUIRED FOR SEMAPHORIN/PLEXIN SIGNALING

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The proper wiring of the nervous system is directed by guidance cues that steer axons along a path of intermediate targets towards the terminal target. Semaphorins serve as repulsive guidance cues that signal through the Plexin/Neuropilin co-receptors to induce growth cone collapse and guidance away from the source of the repellant.

To identify novel downstream effectors of the Semaphorin/Plexin signaling pathway, we took advantage of human umbilical vein endothelial cells (HUVECs) which are easier to culture and transfect compared to primary neurons. Furthermore, HUVECs endogenously express the PlexinD1 receptor and respond to Semaphorin3E by collapsing the cell body. In an siRNA screen using a library of 227 human small G proteins, GAPs, and GEFs, we identified 21 genes whose depletion reduced this collapse phenotype in the presence of Sema3E. In a secondary screen, we found that siRNA-mediated depletion of 10 of these 21 genes in HUVECs also reduced the Sema3E-dependent reduction in migration. By depleting each of these 10 genes in primary mouse dorsal root ganglion (DRG) neurons, we determined that ArfGAP1 is required for Sema3A-induced growth cone collapse. We find that treatment of DRG cultures with QS11, a small molecule inhibitor of ArfGAPs, phenocopied the reduction of Sema3A-dependent growth cone collapse due to ArfGAP1 depletion. This effect is specific as QS11 does not affect EphrinA5-induced growth cone collapse. ArfGAP1 is a GTPase activating protein for the Arf family of small G proteins which functions in cell migration, receptor trafficking, and vesicular transport. By identifying the cognate Arf that functions in growth cone collapse, we aim to biochemically dissect the pathway downstream of Semaphorin/Plexin signaling that proceeds through ArfGAP1.

THE COXSACKIEVIRUS AND ADENOVIRUS RECEPTOR (CAR) IS INVOLVED IN SYNAPTIC TRANSMISSION.

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The Coxsackievirus and Adenovirus Receptor CAR is a transmembrane adhesion protein highly expressed in the embryonic heart and brain development. During the past years different CAR knockout (KO) mice were established to investigate CAR's function in vivo. The conventional CAR KO is embryonic lethal with intracardial bleeding in mid gestation. The inducible heart specific CAR KO mice are viable but develop a conduction defect, which prompted us to investigate a potential role for CAR in neuronal conduction. Available data show CAR expression in neurons and glia but do not yet fully describe the function of CAR in brain. To analyse the role of CAR in the CNS we developed a brain specific CAR KO using the Cre lox recombination system. The KO animals are viable and fertile and do not show any obvious morphological changes in the brain. We investigated expressional changes of various genes in the CAR KO compared to control brains and found an upregulation of Synaptotagmin 2 (Syt2) in the hippocampus. Syt2 is a calcium sensor that binds phospholipids in a calcium dependent manner and is involved in neural exocytosis. Thus we investigated synaptic vesicle release in CAR KO hippocampal neurons and controls and documented changes in the release after membrane depolarization. To further elucidate the role for CAR in synaptic transmission, we investigated if the altered exocytosis leads to changes in electrical activity in brain slices. Our electrophysiological analysis revealed an increased electrical transmission in KO hippocampus and increased induction of long term potentiation as compared to control animals.
Thus our data indicate a so far unknown role of the Coxsackievirus and Adenovirus Receptor in synaptic transmission.

THE PLEXIN B RECEPTOR INTEGRATES BOTH ATTRACTIVE AND REPULSIVE SEMAPHORIN-MEDIATED GUIDANCE IN *DROSOPHILA* TO ENSURE ACCURATE CNS CIRCUITRY ASSEMBLY

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Longitudinal axon fascicles within the CNS provide neuronal connections between body segments, resulting in coordinated neural signaling along the anterior-posterior axis. We find that the secreted semaphorins semaphorin-2a (Sema-2a) and semaphorin-2b (Sema-2b) act together to regulate select longitudinal tract formation in the *Drosophila* embryonic CNS. Both Sema-2a and Sema-2b utilize the same neuronal receptor, plexin B (PlexB), but mediate distinct guidance functions. Sema-2b is selectively expressed in a subset of anteriorly projecting interneurons and is required for these axons to recognize and fasciculate with each other to establish a specific longitudinal pathway within the CNS neuropil. Other longitudinal tracts within this same region, including the intermediate Fasciclin-II^{+} (FasII) tract, also require Sema-2b for their organization. Sema-2b attraction also promotes the subsequent CNS targeting of chordotonal sensory axons to the intermediate FasII tract: therefore, Sema-2b is required to assemble both a subset of CNS interneurons and also the sensory afferents that form synapses within this restricted region. In the absence of Sema-2b, the organization of these CNS longitudinal connectivities is severely disrupted, as are chordotonal synaptic contacts. This produces strong deficits in larval behaviors normally dependent upon chordotonal sensory function. In contrast to Sema-2b, Sema-2a is more broadly expressed within the CNS and signals repulsion to keep these tracks in appropriate positions within the CNS. Both Sema-2a repulsion and Sema-2b attraction are transduced through the same receptor, PlexB, in these projections. Taken together, these results reveal a common PlexB-mediated guidance mechanism underlying both the formation of select CNS longitudinal tracts and the acquisition of appropriate sensory input required for functional neural circuit assembly.

DEGENERATING TRACTS DO NOT PROVIDE A STRONG GUIDANCE CUE FOR REGENERATING OPTIC AXONS: OBSERVATIONS IN THE *ASTRAY/ROBO2* MUTANT

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During formation of the optic projection in *astray/robo2* mutant zebrafish, optic axons exhibit rostro-caudal projection errors, ectopic midline crossing and increased arbor termination area. Here we show that many of these errors persist into adulthood. Even when Robo2 function is conditionally reduced only during initial formation of the optic projection, rostral projection errors are retained in adults. Adult errors include massive ectopic optic tracts in the telencephalon. During optic nerve regeneration in *astray/robo2* animals, these tracts are not re-populated and ectopic midline crossing is reduced compared to unlesioned mutants. However, other errors, such as expanded termination areas and ectopic growth into the tectum, are recommitted by regenerating optic axons. Ubiquitous overexpression of Slit2 during regeneration does not elicit major pathfinding phenotypes. This shows (1) the absence of an efficient correction mechanism for large-scale projection errors of optic axons during development, (2) that degenerating tracts do not provide a strong guidance cue for regenerating optic axons in the adult CNS, and (3) a reduced importance of Robo2 and its ligand, Slit2, for pathfinding of regenerating optic axons relative to development.
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HIGHWIRE AND DOWNSTREAM WND/JNK SIGNALING REGULATE AXONAL OUTGROWTH AND BRANCHING IN GLUTAMATERGIC NEURONS IN THE BRAIN

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Establishment of appropriate neural connections is a fundamental process in development. At the larval NMJ, a conserved E3 ubiquitin ligase, Highwire (Hiw), regulates the morphology of the axon terminus by down-regulating a conserved MAP Kinase Kinase Kinase Wallenda (Wnd) and downstream JNK signaling. In larval motoneurons, this pathway is a potent regulator of bouton growth and nerve terminal branching. Here we investigate the function of this pathway in other neurons in the brain, and find a remarkable phenotype in optic lobe pioneer (OLP) neurons. Three of these neurons, labeled by PDFr-GAL4, normally project in a tightly fasciculated bundle towards the larval optic neuropil. Misregulation of Wnd signaling in these neurons, either through mutation of hiw or overexpression of wnd, causes a dramatic axonal overgrowth, overbranching and mis-projection defect, where axon branches exit the brain and grow along the Bolwig's nerve into the eye imaginal disc. Similar to the NMJ, this phenotype requires downstream factors JNK and Fos. When the eye disc is depleted, mutant OLP axon branches meander in the brain lobe. We interpret that Hiw and Wnd regulate propensity for axonal growth rather than specific guidance decisions in these neurons.

Of the three OLP neurons, only one is glutamatergic. Interestingly, all of the mis-projecting axon branches arise from this single glutamatergic neuron, while a cholingeric OLP neuron projects normally. Markers for nuclear signaling downstream of Wnd suggest that the Wnd pathway is active and highly regulated specifically in the glutamatergic OLP neuron, but not the cholinergic neuron. This specificity for the glutamatergic neuron is intriguing, since Drosophila motoneurons are also glutamatergic. These observations suggest that the Wnd pathway regulates axon morphology in a specific subset of cell types.

An essential component of glutamatergic neurons is the vesicular glutamate transporter VGLUT. The single Drosophila transporter, DVGLUT, is transcriptionally down-regulated when Wnd signaling is over-active. Furthermore, we find that over-expression of DVGLUT leads to activation of Wnd signaling, and promotes overgrowth of the glutamatergic OLP neuron. These results suggest that Wnd functions in a feedback loop that regulates the levels of DVGLUT. Because DVGLUT levels directly affect glutamatergic transmission, our findings suggest the existence of a common pre-synaptic mechanism for regulating both synaptic function and structure.

FLRT2 AND FLRT3 ACT AS REPULSIVE GUIDANCE CUES FOR UNC5-POSITIVE NEURONS

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Development of the layered mammalian neocortex happens 'inside-out' such that late born neurons in the ventricular zone migrate through older neurons and occupy more superficial layers in the cortical plate (CP). However, a population of upper layer neurons expressing Svet1/Unc5D pauses in the subventricular zone (SVZ) and have a delayed migration towards the CP. The underlying mechanism of this behavior is unknown. The Netrin receptor Unc5B has recently been shown to bind fibronectin and leucine-rich transmembrane protein-3 (FLRT3), but the topography and function of this interaction in neuronal development is unknown. We show that the ectodomains (ECDs) of all three FLRT proteins (FLRT1-3) are cleaved in the juxtamembrane region by metalloproteases. Soluble FLRT ECDs bind Unc5 receptors (in *trans*) with high and specific affinities. FLRT2 binds Unc5D with highest affinity, whereas FLRT3 binds Unc5B with highest affinity. Soluble FLRT2/3 ECDs induce growth cone collapse of Unc5+ cortical neurons and repel axons and soma of hippocampal neurons. In vivo, FLRT2 and Unc5D regulate radial migration of a subset of cortical neurons. When Unc5D+ cells reside in the SVZ, FLRT2 is strongly expressed in the CP. In FLRT2-/- and Unc5D-/- embryos, some SVZ cells enter the intermediate zone prematurely, whereas overexpression of Unc5D delayed the migration of SVZ neurons to the CP. These results suggest that FLRT2 and Unc5D regulate the migration of a subset of SVZ cortical projection neurons, consistent with FLRT2 acting non-cell-autonomously as a repulsive cue for Unc5D+ cells *in vivo*.

BMP RECEPTOR SIGNALING REGULATES AXON OUTGROWTH THROUGH THE LIMK1/COFILIN PATHWAY

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As a growth cone extends through the constantly changing embryonic environment, it makes stereotyped directional decisions based on the presence of extrinsic guidance cues. By additionally regulating rate of axon outgrowth, the growth cone will thus receive directional information at particular speed or stage of development. In our previous work, we have identified the key intracellular effectors that permit the Bone Morphogenetic Proteins (BMPs) to regulate the rate of commissural axon outgrowth in the developing spinal cord. BMP binding results in the upregulation of Lim kinase 1 (Limk1) activity, thereby inactivating cofilin, a direct regulator of actin polymerization. Our studies have shown that modulating the balance between the activities of Limk1 and cofilin has profound effects on commissural axon growth. The constitutive activation of Limk1 in embryonic chick commissural neurons causes commissural axons to stall. In contrast, the downregulation of Limk1 or over expression of cofilin in commissural neurons accelerates axon outgrowth.

How is the regulation of Limk1/cofilin mediated by the BMP ligands? Our studies have shown that type IB BMP receptor (BmprIb) is the critical receptor that translates the activities of the BMP guidance signal in commissural neurons. However, although previous studies have suggested a key role for the type II BMP receptor in regulating the activity of Limk1, the contribution of the type I BMP receptors to this signaling mechanism has remained unclear. Here, we show a critical role for BmprIb in regulating commissural axon outgrowth by inactivating cofilin. BmprIb is required for the ability of the BMPs to inactivate cofilin: BMP stimulation of dissociated commissural neurons deficient for *BmprIb* does not result in phosphorylated cofilin, in contrast to what is observed with wild type neurons. Moreover, modulating BmprIb activity levels *in vivo* results in axon outgrowth phenotypes similar to those observed after modulating Limk1 activity. Taken together, these results suggest that BmprIb is a critical component of the receptor complex that controls the rate of commissural axon outgrowth through the dorsal spinal cord, thereby regulating the developmental stage and/or speed at which subsequent directional and growth information is received.

CHARACTERIZING THE MOLECULAR MECHANISMS OF ACTION OF THE AXON GUIDANCE RECEPTOR PLEXIN A

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Neuronal connectivity is precisely determined by axonal pathfinding during developmental stages. The growing tip of these axons, the growth cone, detects, via axon guidance receptors, a wide range of attractive and repellent environmental cues that function over both long and short range. These antagonistic cues are then integrated within the growth cone to modulate the actin cytoskeleton and cell-cell/cell-substrate adhesion, thereby specifying the direction of axon extension. One goal of our studies is to understand the molecular mechanisms by which these attractive and repulsive cues are integrated. To do this, we have utilized the Drosophila nervous system and initially concentrated our research on one of the most widely expressed families of axon guidance receptors, the Plexins, which serve as functional receptors for members of one of the largest family of axon guidance cues, the Semaphorins. Our recent results reveal that Plexin associates with the multidomain Redox enzyme MICAL, which is necessary for specific Plexin-mediated actin rearrangements in vivo. Interestingly, the intracellular region of Plexins also contains a Ras GTPase activating protein (GAP) domain that is divided into two segments by a Rho family GTPase-binding domain (RBD). However, the in vivo significance of the Plexin GAP domain in axon guidance and actin cytoskeletal rearrangements has not been examined. Therefore, to begin to examine the in vivo significance of the Plexin GAP domain, we have performed structure-function analysis of the cytoplasmic portion of the Plexin receptor. Our results reveal that the intracellular portion of the plexin receptor is required for specific plexin-mediated axon guidance events in vivo. Likewise, we also find that conserved amino acid residues within the GAP domain that are predicted to be critical for Plexin GAP activity are also necessary in vivo for Plexin-mediated axon guidance. Ongoing work is aimed at identifying the specific small GTPases whose activity Plexin A regulates in vivo and specific ways by which the activity of this Plexin GAP domain may be regulated.

MODULATION OF AXONAL REPULSIVE RESPONSE: NOVEL FUNCTION FOR TRANSMEMBRANE SEMAPHORIN AS *CIS* INHIBITOR

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The correct navigation of axons to their targets depends on guidance molecules in the extracellular environment. The neuronal expression patterns of axonal guidance receptors largely predict their responsiveness to specific guidance cues. However, it is clear that additional layers of regulation enable the neuron to modulate responses in spatial and temporal manners.
DRG sensory neurons express the Semaphorin6A (Sema6A) receptor Plexin-A4, but barely respond to exogenous Sema6A. Remarkably, ablation of Sema6A in these neurons enhanced their responsiveness to external Sema6A in a Plexin-A4 dependent manner. Using heterologus systems we found that the co-expression of Sema6A and Plexin-A4 hinders the binding of exogenous ligand; suggesting that a Sema6A-Plexin-A4 *cis* interaction serves as an inhibitory mechanism. Finally, we provide evidence for differential modes of interactions of Sema6A with Plexin-A4 in *cis* versus in *trans*. This may ensure that *cis* interaction will not lead to constitutive activation of the receptor.
Overall our results show that co-expression of a transmembrane cue together with its receptor can serve as a guidance response modulator, enabling the axons to navigate through an otherwise repulsive environment

MOLECULAR MECHANISMS OF DENDRITE TARGET RECOGNITION

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The precision with which neurons form synaptic connections is remarkable. How dendrites recognize and form synaptic connections with their specific targets is unclear. Our goal is to study the connections formed by an identified neuron, L4, in the lamina of the fly visual system. L4 dendrites selectively form synapses with a specific target, the L2 neuron, but not with that of a highly related immediately neighboring neuron, L1. Each L4 neuron extends 3 dendrites: one remains within the same cartridge, while the others project into two neighboring posterior cartridges. The asymmetry in dendrite structure of L4 may underlie selective motion detection. L4's binary choice in synaptic target selection and the availability of cell-specific genetic tools provide for a simple system to uncover general principles that regulate dendrite target recognition. Here I describe an approach to dissect the mechanisms by which the motion sensitive circuitry is established in *Drosophila*.

ENDOCYTOSIS OF EPHA RECEPTORS IS ESSENTIAL FOR THE PROPER DEVELOPMENT OF THE RETINOCOLLICULAR TOPOGRAPHIC MAP

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Endocytosis of Eph-ephrin complexes may be an important mechanism for converting cell-cell adhesion to a repulsive interaction. Here we show that an endocytosis-defective EphA8 mutant forms a complex with EphAs and blocks their endocytosis in cultured cells. Further, we used bacterial artificial chromosome transgenic mice to recapitulate the anterior > posterior gradient of EphA in the superior colliculus (SC). In mice expressing the endocytosis-defective EphA8 mutant, the nasal axons were aberrantly shifted to the anterior SC. In contrast, in transgenic mice expressing wild type EphA8, the nasal axons were shifted to the posterior SC, as predicted for the enhanced repellent effect of ephrin-A reverse signaling. Consistent with these results, nasal axons exhibited opposite growth patterns in the presence of wild type and endocytosis-defective EphA8 cells. In addition, we found that Rac activity was significantly reduced in the SC expressing the endocytosis-defective EphA8 mutant. Consistently, the retinal axons demonstrated a good growth preference for cells expressing both EphA8 and dominant negative Rac (N17Rac), whereas they did a significant avoidance of cells expressing EphA8 alone. Importantly, expression of dominant negative Rac in the anterior SC revealed that the terminations of nasal axons in the SC were expanded anteriorly, similar to what we have observed in mice expressing the endocytosis-defective EphA8 mutant. Taken together, these results indicate that endocytosis of the Eph-ephrin complex is a key mechanism by which axonal repulsion is generated for proper guidance and topographic mapping.

MICAL AND SEMAPHORIN/PLEXIN-MEDIATED ACTIN REARRANGEMENTS IN VIVO

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Semaphorins are one of the largest families of extracellular guidance cues and play critical roles in axon guidance, dendrite morphology, and spine plasticity. Semaphorins direct these neuronal behaviors by eliciting destabilizing effects on F-actin in a localized manner that include a loss of F-actin, the decreased ability to polymerize new F-actin, a decrease in the number of F-actin bundles, and the regulation of F-actin-rich filopodia/branches. However, despite significant progress in the identification of Semaphorin (Sema) receptors and their signaling pathways, the molecules linking them to the precise control of these cytoskeletal rearrangements have remained a mystery. Recently, we and others have found that members of the phylogenetically conserved MICAL family of oxidoreductase (Redox) enzymes associate with the cytoplasmic portion of plexins, which are large cell surface semaphorin receptors, and mediate axon guidance, synaptogenesis, dendritic pruning, and other cell morphological changes. One of the questions that has emerged from this work with MICAL is what is the cell biological role of MICAL and our results now reveal that MICAL regulates the organization of the actin cytoskeleton in vivo. We find that MICAL proteins colocalize with F-actin in vivo and are necessary for normal F-actin organization and bundling during development. Elevating MICAL levels in vivo is sufficient to disassemble bundles of actin filaments and reorganize parallel F-actin bundles into a meshwork of short, branched actin filaments. Moreover, our results point to a model in which Sema/Plexin/MICAL signaling directly destabilizes F-actin, which triggers a secondary response that produces branched meshwork actin and actin-rich extensions. Interestingly, these observations provide a more complete understanding of the roles of repulsive guidance cues in vivo. Therefore, we propose that repellents such as semaphorins disassemble or “prune back” the actin network in vivo, and this “pruning” process initiates a secondary cascade of events that enhances cellular complexity/plasticity. These Sema/Plexin/MICAL-induced actin rearrangements would enable navigating axons to identify new, more permissive substrates and could underlie the directional changes associated with Semaphorin repulsive guidance.

BDNF-TRKB REGULATES PALMITOYLATION OF PSD-95 IN DEVELOPING CORTEX THROUGH PLCΓ AND PKMZ

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Post-synaptic density 95 (PSD-95) is the major scaffold for glutamate receptors associating them with their signaling pathways and is therefore critical for brain maturation, learning and memory. In the rodent visual pathway, synaptic PSD-95 levels increase within hours of eye opening EO, pattern vision onset, and changes in visual circuit refinement. PSD-95 requires palmitoylation for transport to, and insertion at synapses. This lipid modification of synaptic proteins is critical to brain maturation and plasticity but the initiating signals are largely unknown making it difficult to identify primary causes of synaptic dysfunction and brain disease. BDNF-TrkB signaling and PKMζ; are also critical for synapse maturation, late long-term potentiation (LTP) and maintenance of long-term memory in cortex. Previously, we showed that NMDA glutamate receptors activate BDNF-TrkB signaling through the Phosphoinositide 3-kinase and Akt to initiate synaptic transport of PSD-95 in vitro.
To study trafficking of PSD-95 in vivo, we have used TrkB ^{F616A} mice, which have been engineered so that a chemical compound 1NM-PP1 binds to this mutation site and blocks the activation of TrkB. Using Elvax, a slow release polymer, we have applied 1NM-PP1 to the surface of visual cortex. We have found that BDNF-TrkB signaling is necessary for the developmental increase of PSD-95 at visual cortical synapses upon eye opening. We also show that TrkB activity is necessary for palmitoylation of PSD-95 via Phospholipase Cγ; (PLCγ) and PKMζ. Furthermore, we demonstrate that transport of PSD-95 to visual cortical synapses after eye opening is suppressed by blockade of PKMζ.
Our data suggest that the BDNF-TrkB signaling regulate palmitoylation of PSD-95 through PLCγ to PKMζ; and that this pathway underlie formation of the visual cortical circuitry during development as well as late LTP and maintenance of long-term memory.

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THE CHEMOTACTIC RESPONSE OF AXONS SCALES LINEARLY WITH GRADIENT STEEPNESS

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Molecular gradients provide important guidance information for developing axons *in vivo*. A quantitative understanding of how such gradients cause axons to follow particular trajectories requires knowledge of how the chemotactic response of axons varies with gradient parameters. A recently-proposed Bayesian model for how axons combine noisy receptor-binding measurements to determine gradient direction predicts that, for shallow gradients, the chemotactic response should scale linearly with gradient steepness (defined as fractional change per unit distance). Here we test this prediction using an assay which allows precise control over gradient conditions. The results confirm that the response drops linearly to zero as gradient steepness is decreased. Besides providing further support for the model, this result also argues against there being an absolute threshold in the size of the gradient signal below which gradient detection is impossible.

AUTOINHIBITION AND ACTIVATION MECHANISMS OF THE PLEXIN INTRACELLULAR REGION

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Plexins are cell surface receptors that bind their semaphorin ligands and transduce signals for regulating neuronal axon guidance and many other important processes. The intracellular domain of plexins contains a Ras GTPase activating protein (GAP) domain that is essential for signaling. Previous studies have shown that the GAP activity of plexins is controlled by both semaphorin binding to their extracellular region and RhoGTPase binding to the intracellular region. Our recent crystal structure of the plexin A3 intracellular region has demonstrated that the GAP domain of plexin adopts a more closed conformation compared to canonical RasGAPs, indicating an autoinhibited state of the plexin GAP that cannot catalyze GTP hydrolysis. We have now determined another crystal structure of the plexin intracellular region, which adopts a different conformation compared to the original structure, although the GAP domain remains in the inactive conformation. In addition, this structure suggests an inactive dimer that may help further stabilize the inactive state of plexin on cell surface. Our structure-based mutational analyses have suggested an allosteric network in the plexin intracellular region that likely underlies the activation mechanism of the GAP domain. Using in vitro biochemical assays, we show that the plexin GAP domain is indeed autoinhibited, and the GAP activity can be activated dramatically through an allosteric mechanism.

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INTRINSIC B-RAF KINASE SIGNALING DICTATES MORPHOGENESIS AND SPINAL PROJECTIONS OF SENSORY NEURONS

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Extracellular signals as well as cell-autonomous genetic programs are required for the precise wiring of neuronal circuits during development. The molecular mechanisms of how the genetic programs are regulated and the precise roles of intracellular signal transduction pathways in this process, however, remain elusive. The MAP kinase pathway is a major conduit that translates extracellular stimuli into intracellular responses. Previous loss-of-function studies have shown that the serine/threonine kinase RAF, an upstream activator in the MAP kinase cascade, is essential for neurotrophin-induced sensory axon growth *in vitro*, as well as target innervation *in vivo*. To gain insight into the role RAF signaling plays in neural development, we used a nestin-Cre line to selectively express in neurons a constitutively active form of B-RAF (caB-RAF) from its endogenous locus. caB-RAF robustly activates MEK and ERK *in vivo* and leads to profound defects of both proprioceptive and nociceptive afferents. Instead of terminating in the superficial layers in the dorsal horn as is the case in wild type littermates, the TrkA-positive nociceptive afferents expressing caB-RAF aberrantly project into deeper dorsal spinal cord, with many processes crossing the midline. Proprioceptive sensory axons normally enter the spinal cord at tightly restricted medial dorsal root entry zones. caB-RAF causes proprioceptive axons to enter the spinal cord across the entire dorsal surface of the cord and impinge into and traverse the target zone of nociceptive afferents. And while caB-RAF-expressing proprioceptive processes still extend into their canonical target area in the middle and ventral spinal cord, our data suggest that the caB-RAF-expressing afferents overcome the resident inhibitory signals via activation of a cell-autonomous intrinsic growth program. Finally, we also observe that the caB-RAF:nestin-Cre mice exhibit macrocephaly as well as hydrocephalus, which notably phenocopies two hallmarks of the human neuro-cardio-facio-cutaneous syndromes.

MECHANISMS OF AXON PATHFINDING IN ZEBRAFISH.

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To reveal a specific programme for zebrafish motor axon pathfinding we established a method to selectively block motor axons pathfinding by interfering with LIM domain transcription factor signaling. DD domain dimerization of CLIM can activate LIM-HDs and downstream gene-transcription while over-expression of dominant-negative CLIM (DN-CLIM), which lacks the DD domain, blocks LIM-HD activity. Motor axons in DN-CLIM injected HB9:GFP transgenic zebrafish, in which motor neurons fluoresce, are unable to exit the spinal cord. However, GFP expression and expression of other motor neuron markers are retained. This provides us with an excellent research model to find genes involved in motor axon pathfinding downstream of LIM-HDs. Gene array expression profiling was carried out on GFP+ motor neurons by fluorescence-activated flow sorting (FACS) with and without prior injection of DN-CLIM mRNA to elucidate the potential genes relevant to motor axon pathfinding. Genes that were most strongly down-regulated in DN-CLIM injected embryos were considered to belong to a motor axon specific growth programme and their expression was verified by qPCR and by in situ hybridization. There were around 170 genes at least 2-fold down-regulated in DN-CLIM mRNA injected embryos. Of these genes, 30 genes were specifically enriched in motor neurons as determined by cross-correlating the resulting list of genes with an expression profile for motor neurons (GFP+ vs. GFP- cells). Calca, tac-1 and nrm1(for novel recognition molecule 1) genes were retrieved and showed specific expression pattern in motor neuron and obvious down-regulation after DN-CLIM injection by in situ hybridization. This validated the screen results. Nrm1 contains a C-type lectin domain representing a potential cell surface receptor for guidance factors. The promoter region contains multiple recognition motifs for the LIM-HDs islet-1 and lhx3, which suggests that the gene could even be a direct target of LIM-HDs. Gene knock-down experiment with two independent morpholinos led to stalling of 97% of all CaP motor axons. Overall development was not retarded as determined by normal migration of the lateral line primordium. This demonstrated that this novel gene can specifically affect motor axons guidance in zebrafish. Analyses of phenotypes (growth cone dynamics; synapse formation) and molecular properties of nmr1 are in process. These findings indicate that using a gene array of FAC-sorted motor neurons from the developing zebrafish spinal cord provides a good method to identify the gene expression programme specific for motor axon pathfinding.

REGENERATION OF AXONS IN INJURED SPINAL CORD INDUCED BY BMP4 ACTIVATION IN ADULT SENSORY NEURONS

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Diminished axon growth amplitude in mature neurons poses an obstacle for axon regeneration. One of the challenges in spinal cord injury (SCI) research, therefore, is to define molecular pathway that can rejuvenate the axon regenerative capability of injured neurons and to develop therapeutic strategies to turn it on. We optimized a minimally invasive technique to deliver adeno-associated virus (AAV) directly into lumbar CSF space and demonstrated that dorsal root ganglion (DRG) neurons were selectively transduced with high efficiency. Activation of the BMP4 signaling in DRG neurons by way of an AAV vector encoding BMP4 resulted in robust sensory axon regeneration after SCI. The promoting effect of the AAV-BMP4 seems to be greater than that of AAV-Smad1, a transcription factor that mediates BMP4 signaling, suggesting that BMP4 not only acts through a cell-autonomous mechanism to enhance the axon growth potential of adult DRG neurons, as demonstrated in neurite outgrowth assay that DRG neurons from AAV-BMP4 injected mice extended longer axons than control DRG neurons from AAV-GFP, but also through cell non-autonomous mechanisms. We have evidence to suggest that astroglial reactivity was attenuated at the injury site in AAV-BMP4 injected mice. We further show that transected axons were also able to regenerate across the lesion site even when the virus was delivered after the injury has occurred, therefore mimicking clinical scenario. Thus, our results point to a new therapeutic approach to promote axon regeneration after SCI injury. Manipulation of the BMP/Smad1 pathway through intrathecal delivery of AAV can be readily tested in clinical setting.

WNT-PLANAR CELL POLARITY SIGNALING CONTROLS THE ANTERIOR-POSTERIOR ORGANIZATION OF MONOAMINERGIC AXONS IN THE BRAINSTEM

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Monoaminergic neurons (serotonergic (5HT) and dopaminergic (mdDA)) in the brainstem project axons along the anterior-posterior (A-P) axis. Despite their important physiological functions and implications in disease, the molecular mechanisms that dictate the formation of these projections along the A-P axis in vivo remain poorly understood. Here we reveal a novel requirement for Wnt/PCP signaling in the A-P organization of both the 5HT and mdDA systems. We find that 5HT and mdDA axons express the core PCP components Frizzled3, Celsr3 and Vangl2. In addition, both ascending and descending projections show A-P guidance defects in Frizzled3, Celsr3 and Vangl2 mutant mice. The only known ligands for PCP signaling are Wnt proteins. Wnt5a and Wnt7b attract or repel 5HT and mdDA axons in vitro and are expressed in gradients along the A-P axis of the brainstem, consistent with their role as directional cues. In addition, Wnt5a mutants show transient A-P guidance defects in mdDA projections. We furthermore observe that the cell bodies of migrating descending 5HT neurons eventually re-orient along the direction of their axons. In Frizzled3 mutants, many 5HT and mdDA neuron cell bodies are oriented abnormally along the direction of their aberrant axon projections. This finding demonstrates that A-P axon guidance by Wnt/PCP signals is essential for the proper A-P cellular organization of monoaminergic nuclei, and this is likely to apply to other neural systems. In all, this study identifies Wnt/PCP signaling as a global A-P guidance mechanism that controls axonal and cellular organization beyond the spinal cord.

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SEMA6A-PLXNA2 INTERACTIONS INITIATE BIDIRECTIONAL SIGNALLING

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Interactions between the vertebrate class 6 semaphorin, Sema6A and the class A plexins, PlxnA2 and PlxnA4, play crucial roles in the development of the mammalian brain, for example in the lamination of mossy fibres in the hippocampus, the migration of cerebellar granule cells and the guidance of thalamocortical and corticospinal axons. In many of these processes, Sema6A has been shown to act by promoting signalling through PlxnA2 and/or A4 receptors. Nevertheless, phenotypic observations in Sema6A knock-out mutant mice have suggested that Sema6A-expressing cells, such as neurons from dorsal thalamus and cerebellum, may also exhibit cell-autonomous defects, suggesting that Sema6A may also act as a receptor.

To investigate the possibility of bidirectional signalling and the biochemical mechanisms of reciprocal activation we have characterised the interaction between the extracellular domains of Sema6A and PlxnA2 in crystal structures. These structures reveal that two PlxnA2 monomers interact, independently, with the two monomers of a Sema6A dimer. Binding to Sema6A does not induce a conformational change in the PlxnA2 extracellular domain examined, and while monomerised Sema6A can bind PlxnA2 it is incapable of initiating signalling. These data favour a model of semaphorin-induced plexin dimerisation, possibly followed by higher-order clustering, as the mechanism of activation.

Using an in vitro assay of cerebellar granule cell migration, we demonstrate that the Sema6A-PlxnA2 interaction can stimulate signalling in either direction. The migration of wild-type granule cells is retarded when they are grown on NIH3T3 cells expressing full-length Sema6A, and PlxnA2-/- granule cells are insensitive to this effect. Conversely, PlxnA2 expressed on 3T3 cells promotes migration of granule cells. Sema6A-/- cells are unresponsive to PlxnA2 and this effect is not seen on cells expressing a mutant form of PlxnA2 that does not bind Sema6A. Additional effects of signalling in both directions are observed in dissociated neurons on axonal and dendritic morphogenesis. These results demonstrate a bidirectional interaction between Sema6A and PlxnA2 in neurons and provide a biochemical context in which to interpret complex in vivo phenotypes across several developmental systems.

THE INTRACELLULAR DOMAIN OF FRAZZLED CAN TRANSLOCATE TO THE NUCLEUS AND REGULATE TRANSCRIPTION OF *COMMISSURELESS* TO PROMOTE MIDLINE CROSSING

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In bilaterally symmetric animals, attractive and repulsive axon guidance must be coordinated at the midline to generate a functional nervous system. The ligands and receptors that control midline axon guidance are conserved in vertebrates and invertebrates. In the embryonic Drosophila CNS, most axons project contralaterally, traveling through the midline, which expresses the repellent Slit. During the period of time when commissural neurons are sending their axons across the midline, sensitivity to Slit is reduced by expression of Commissureless (Comm), which sorts the Slit receptor Roundabout into endosomes, reducing its expression on the surface of the growth cone. Previously, we have shown that the attractive axon guidance receptor Frazzled (Fra), the Drosophila homolog of DCC, activates *comm* transcription, but the mechanism remains unknown. Recent data suggests that Fra may induce *comm* expression by functioning as a transcriptional activator. When expressed in Drosophila cells *in vitro* or neurons *in vivo*, the intracellular domain of Fra (FraICD) is localized in nuclei. Nuclear localization of the FraICD correlates with *comm* expression *in vitro* and expression of the FraICD in a subset of ipsilateral neurons is sufficient to induce ectopic *comm* expression and midline crossing. Expression of the FraICD in a subset of commissural neurons partially rescues the midline crossing defects of *fra* mutants. We present a model in which Fra is proteolytically processed, releasing a cytoplasmic domain that translocates to the nucleus to either directly or indirectly activate *comm* transcription and promote midline crossing.

GDNF ACTS AS A CHEMOATTRACTANT TO SUPPORT EPHRINA-INDUCED REPULSION OF LIMB MOTOR AXONS

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Genetic studies in vertebrates have shown that two ligand/receptor systems, ephrinA/EphA4 and GDNF/Ret, cooperate in guiding lateral motor column (LMC_L) axons towards the dorsal trajectory in the hindlimb. While the role of ephrinAs as repulsive cues is well established, it has remained unclear how GDNF acts on LMC_L axons to promote dorsal pathway selection. We tested three possible roles for GDNF in this system: (1) GDNF may enhance the ability of EphA4 to signal repulsion; (2) GDNF may promote growth of LMC_L axons, while EphA4 provides instructive signaling to avoid the ventral limb; (3) GDNF is a chemoattractant that guides axons towards the dorsal limb, while ephrinAs repel them from the ventral limb. We show that GDNF/Ret does not positively modulate ephrinA/EphA4 signaling, because the addition of GDNF attenuated ephrinA-induced growth cone collapse in LMC explant cultures, and this effect required Ret activation. Moreover, the sensitivity to ephrinAs was not changed in Ret-deficient explant cultures, and there was no difference in EphA4 phosphorylation between Ret knockout and wild-type spinal cords. To test if GDNF acts as a growth-promoting factor for motor axons in the context of ephrin/Eph engagement, we performed time-lapse imaging of motor neurons co-cultured with ephrinA-expressing HeLa cells and observed that GDNF reduces growth cone collapse and retraction and facilitates growth cone recovery after contact with ephrinA5. Moreover, overnight stimulation of dissociated LMC cultures with GDNF increased axon length. In addition, we demonstrate that GDNF acts as a true chemoattractant, because LMC_L axons showed attractive turning in a stable GDNF gradient, and this effect was Ret-dependent. Our findings establish a new role for GDNF and reveal a requirement for an attractive and a repulsive guidance cue acting simultaneously to ensure correct motor axon pathfinding at an intermediate choice point.

MAPPING THE DYNAMICS OF OCULOMOTOR NERVE PROJECTIONS TO THE EXTRAOCULAR MUSCLES IN THE ZEBRAFISH AND THE ROLE OF A2-CHIMAERIN.

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In vertebrates, eye movements are controlled by six extraocular muscles, innervated by three cranial nerves – the oculomotor, the trochlear and the abducens. Studies in the chick embryo have shown that the oculomotor nerve (OMN) undergoes a stereotyped pattern of outgrowth and branching to its four muscle targets[1]. Perturbations of this wiring pattern in humans give rise to congenital eye movement disorders such as Duane's Retraction Syndrome (DRS), which can arise due to mutations in the RacGAP signalling molecule α2-chimaerin[2]. However, the dynamics of axon behaviour which govern topographic axon projections in the ocular motor system have not been characterised, nor has the role of α2-chimaerin been elucidated. We have therefore used the zebrafish model system to study the developmental dynamics of axon guidance to the extraocular muscles, and the role of α2-chimaerin in this process.

We have used two-photon time-lapse imaging of the *Isl1-GFP* transgenic zebrafish line to map the normal development of the OMN and to describe its dynamics at key time-points, e.g. branching decisions. Here we show that the OMN first projects filopodia over a wide area, before restricting protrusions to particular areas of the environment corresponding with muscle anlage. Together with immunostaining of embryos at fixed time points, these movies have also revealed a hierarchical order of appearance of oculomotor axon segments. Mosaic expression techniques to image single GFP-expressing neurons have revealed that axons project from individual OMN subnuclei to muscle targets. This suggests that neuromuscular connectivity is generated by direct axon projections from subnuclei to muscles, rather than axon branching to multiple muscles and subsequent pruning. We have also found that single OMN neurons which express α2-chimaerin harbouring human mutations display extensive filopodial extension and exploratory behaviour as for wild-type axons. However, axons expressing mutant α2-chimaerin do not become restrict their protrusions to select a particular muscle target, resulting in a cell-autonomous stalling phenotype. We are currently creating stable transgenic zebrafish lines expressing mutant forms of α2-chimaerin. This will allow us to use time-lapse imaging to model the human DRS phenotype and to investigate the effects of α2-chimaerin mutations on the entirety of cranial nerve projections to the extraocular muscles.

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14-3-3 PROTEINS REGULATE AXONAL GROWTH CONE RESPONSES BY REGULATING PKA ACTIVITY

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The growth cone is a critical structure regulating the speed and direction of neuronal outgrowth during development. How the growth cone spatially and temporally regulates signals from guidance cues is not fully known. Through a proteomic analysis of a mechanically purified growth cone preparation from E6 chick RGCs we identified several isoforms of the 14-3-3 family of adaptor proteins as major constituents of the growth cone. 14-3-3 proteins bind and regulate the activity of multiple proteins through interactions with phospho-serine and phospho-threonine containing motifs. Using the 14-3-3 antagonist R18 or miRNA-mediated knockdown of individual 14-3-3 isoforms we find that loss of 14-3-3s switch nerve growth factor-dependent repulsion to attraction in E13 chick and P5 rat DRG neurons. This switching effect is blocked by inhibitors of PKA indicating that 14-3-3 proteins may directly regulate PKA. Consistent with this model we find that specific 14-3-3 isoforms interact with the PKA regulatory subunit. Further, we find R18 expression results in a dissociation of the regulatory and catalytic subunit of PKA and increased phosphorylation of the catalytic subunit, indicating increased PKA kinase activity. We are currently examining the effect of 14-3-3 proteins on growth cone cytoskeletal rearrangements using Spatio-Temporal Image Correlation Spectroscopy. We find that 14-3-3 proteins mediate NGF-dependent effects on filopodial length and the retrograde flow of f-actin. Together our data indicates that 14-3-3 proteins play a critical role in modulating growth cone responses to extracellular cues through regulating PKA and associated downstream signaling.

ADF/COFILIN PROTEINS REGULATE THE ACTIN ORGANIZATION AND DYNAMICS UNDERLYING NEURITOGENESIS IN THE DEVELOPING MAMMALIAN BRAIN

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During brain development, initially spherical neurons undergo drastic morphological changes to become the complex, highly branched units of neuronal networks. Studies in cultured neurons and invertebrates have identified signaling proteins that modulate the actin cytoskeleton during neuronal development. However, the physiological role of these proteins during neuronal development and their precise effects on the neuronal cytoskeleton have remained elusive. Here, we analyzed the functional consequences of brain-specific genetic ablation of ADF and cofilin (AC) during in vivo development and in regulating actin dynamics during neurite formation. The ablation of AC proteins resulted in several abnormalities in the developing brain, including a striking decrease in cortical mass and a severe reduction in axonal tract formation. Moreover, AC knockout neurons failed to form neurites. Cytoskeletal aberrations, including increased F-actin, absence of radial actin bundles and filopodia, and irregular looping microtubules in AC KO neurons underlie the failure of neurite initiation. Furthermore, AC KO neurons displayed a complete immobilization of F-actin retrograde flow, indicating that AC proteins are the primary driving force underpinning actin turnover. The exogenous expression of cofilin fully restores neuritogenesis in AC KO neurons, whereas ADF only partially rescues neurites. Using specific activity blocking mutants, we found that although both the F-actin depolymerizing and severing activities of cofilin are necessary for optimal neurite initiation, the severing activity is of greater consequence for neuritogenesis. Taken together, these data suggest that AC proteins regulate neuritogenesis during cortical development.

DECIPHERING THE ROLE OF SPECTRAPLAKINS AS KEY ORCHESTRATORS OF CYTOSKELETAL DYNAMICS IN AXONAL GROWTH

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The mechanisms orchestrating the cytoskeletal dynamics that drive morphogenetic changes of growing axons and dendrites are still little understood. Essential new insights can be obtained from work on spectraplakins, such as mouse ACF7 and *Drosophila* Short stop (Shot). These highly conserved proteins are multi-functional cytoskeletal linker molecules, and in their absence axonal growth and synapse formation are severely impaired. We decipher the mechanistic details of spectraplakin function by utilising Shot which can be studied using efficient fly genetics, both *in vivo* and in our newly established *Drosophila* primary neuron system for the detailed study of cytoskeletal dynamics. We find that Shot acts as a key organiser of both F-actin and microtubule networks. Firstly, Shot regulates filopodia formation in the context of pathfinding, mediated by its EF-hand motifs and their interaction with the putative translational regulator eIF5C. Secondly, Shot is required for the extension of axons, acting to regulate microtubule networks via a number of interdependent molecular mechanisms. Thus, it absolutely requires linkage to F-actin through N-terminal calponin homology domains, and the interaction with microtubules through its C-terminal Gas2 domain and Ctail. We find that Gas2 stabilises MT networks, and that Ctail crucially cooperates with Gas2 to mediate efficient binding along MTs. We identified an independent function of the Ctail, in extending Shot localisation to the growing ends of MTs. This recruitment relies on interaction with EB1 via Ctail's MtLS motifs and is relevant *in vivo*. We provide evidence that, via this mechanism, Shot is able to stabilise and direct MT polymerisation events. Our data demonstrate the function of domains common to all spectraplakins, and significantly contribute to our understanding of neuronal microtubule network regulation at the cellular level.

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Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (********) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64505 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laborator y is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the w aters of the harbor. W ater used for irrigation comes from natural springs and w ells on the property itself. Law ns, trees, and planting beds are managed organically whenever possible. And trees are planted to replac e those felled for construction projects.

Two areas in w hich the Laboratory has focused recent efforts have been those of w aste management and energy conservation. T he Laboratory currently recy cles most w aste. Scrap metal, electronics, construction debris, batteries, fluore scent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream w aste management " system, removing recy clable materials and sending the remaining combustible trash to a cogeneration plant w here it is bur ned to provide electricity , an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced w ith high efficiency fluorescent fixtures, and thousands of incandescent bul bs throughout campus have been replaced w ith compact fluoresc ents. T he Laboratory has also embarked on a project that w ill replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new w ays in which we can reduce our envir onmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. T his book, for example, is printed on recy cled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322